Original Article

Combined Ursolic Acid and Resistance/Endurance Training Improve Type 3 Diabetes Biomarkers-Related Memory Deficits in Hippocampus of Aged Male Wistar Rats

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Abstract

Background: Both aging and diabetes are two well-established risk factors related to type 3 diabetes and memory deficits. Accordingly, diabetes multiplies the effects of aging on cognition impairments once these conditions occur simultaneously. **Methods:** In this present experimental study, 56 male Wistar rats with HFD/STZ-induced T2D were randomized into seven groups (n = eight animals per group): (1) sedentary old non-diabetic (C); (2) sedentary HFD/STZ-induced T2D (D); (3) sedentary HFD/STZ-induced T2D plus UA (UA) (DU); (4) endurance-trained HFD/STZ-induced T2D (DE); (5) resistance-trained HFD/STZ-induced T2D (DR); (6) endurance-trained HFD/STZ-induced T2D plus UA (DEU); and (7) resistance-trained STZ-diabetic plus UA (DRU) rats. Two-way ANOVA was applied to measure the training, supplementation, and interaction effect on serum and gene expression outcomes. **Result:** The study results established no significant interaction effect between the UA supplementation and the resistance/endurance training with regard to the levels of glucose (P = 0.534), insulin (P = 0.327), brain-derived neurotrophic factor (P = 0.191), and insulin-like growth factor-1 (P = 0.448). **Conclusions:** To develop novel practical nutritional strategies involving UA intake, further studies are thus needed to clarify how chronic consumption of UA with/without resistance/endurance training reverses cognition disorder process in old male Wistar rats with HFD/STZ-induced T2D.

Keywords: *Aging, cognition disorders, diabetes mellitus type 2, endurance training, resistance training*

Introduction

Dementia and Alzheimer's disease as neuro-metabolic dysfunctions are examples of these emerging new complications[1] that have a close bidirectional relationship with type 2 diabetes[2] and insulin resistance within the brain.[3] Because of shared mechanisms among T2D and AD, scientists suggested type 3 diabetes.[4]

Several molecular and cellular mechanisms and T3D biomarkers could be molecular factors linking T2D to AD[2,4] due to failure in neuronal glucose uptake in the neurons for energy production.[3,5]

Insulin plays an important indirect neurotrophic role in neuronal survival and synaptic function and plasticity[6] by inducing the expression of other neurotrophic factors such as brain-derived neurotrophic factor,[7] and through its binding to insulin-like growth factor receptors.[8]

BDNF is a protein in the human brain and a member of the neurotrophin family of growth factors which plays an important role in neuronal plasticity and nerve growth during the development and adulthood[9] and a putative mechanism related to physical exercise and cognition is the IGF-1 that seems a fundamental role in neurogenesis seemingly altered in persons with AD.[10]

In this regard, it has been shown that ursolic acid (UA) mediates the cognitive deficits caused by T2D via different cellular and molecular pathways.[11] Studies have shown that UA consumption improves cognitive status in the elderly by improving antioxidant and inflammatory status,[12,13] and improving insulin resistance and glucose metabolism.[14] UA has also been introduced as an anti-atrophic agent that can be effective in preventing the deterioration of various tissues.[12]

Both aerobic and resistance exercise are now recommended as first-line treatment

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for T2D and T3D biomarkers.[15] On the other hand, resistance exercise increases secretion of myokines including IL-6, BDNF, IGF-1, Irisin, and VEGF, of which some, such as IL-6, cross the blood–brain barrier and may mediate neuroplasticity and anti-inflammatory responses in the brain.[16]

In general, the results of meta-analysis performed outside Iran have shown that aerobic intermittent or resistance training can significantly improve the control of glycemic index in patients with type 2 diabetes.[17] Endurance and resistance exercise are one of the contributing factors that both have the same power in controlling the metabolic effects of type 2 diabetes.[18] Aerobic exercise activity has been shown to increase BDNF levels in the elderly, leading to improved brain function including learning and memory,[19] as well as cognitive function and control of food intake and fat and sugar metabolism.[20] Although long-term aerobic exercise has more pronounced effects on BDNF levels, it has been reported that even one session of resistance training increases BDNF.[21] Exercise in old age stimulates growth markers such as IGF-1 in both the whole body and the central nervous system, but accurate information on the duration, intensity, and frequency of exercise during this period is not yet available.[22]

Accordingly, this study aimed to determine the impact of eight weeks of a combination of resistance/endurance exercise training and UA supplementation on T3D biomarkers-related memory deficits in high-fat diet and/or low-dose streptozotocin-induced T2D.

Methods

Animal care, study groups, and ethical approval

All the procedures and experiments corresponded to the relevant directions of the Committee on Animal Research and Ethics at Shahrekord University, Shahrekord, Iran, and developed based on the Guide for Care and Use of Laboratory Animals.

A total number of 56 23-month-old male Wistar rats, weighing 427 ± 44 g, were accordingly purchased from Pasteur Institute, Tehran, Iran, and then maintained at a temperature-controlled facility (20–22°C) with 40–70% humidity, 12-h light/dark cycle, as well as free to access to a commercial standard pellet chow diet and water during the experiments. As the rats became familiarized with the laboratory conditions, they were randomized into seven groups (n = eight animals per group): (1) sedentary old non-diabetic (C); (2) sedentary HFD/STZ-induced T2D (D); (3) sedentary HFD/STZ-induced T2D plus UA (UA) (DU); (4) endurance-trained HFD/STZ-induced T2D (DE); (5) resistance-trained HFD/STZ-induced T2D (DR); (6) endurance-trained HFD/STZ-induced T2D plus UA (DEU); and (7) resistance-trained STZ-diabetic plus UA (DRU) rats. During the research protocol, necessary measures that include animal cages based on groups, numbering and marking of animals, and use of experienced researchers were taken to prevent interchange in the animal from one group to another.

HFD/STZ-induced T2D

As demonstrated in the protocol presented by Zhang *et al*.[23] and Liu *et al*.,[24] HFD/STZ-induced T2D was stimulated. Therefore, the animals in the sham group were fed with a standard rodent-chow diet (sham; energy from fats [10%], carbohydrates [75%], and protein [15%] = 3.8 kcal/g), whereas the animals in the HFD groups were placed on a high-fat diet; that is, HF; energy from fats (55%), carbohydrates (31%), as well as protein (14%) = 5.2 kcal/g. Then, an eight-week diet was considered for maintaining the two groups. In the course of the fourth week, the HFD/STZ-induced T2D group was treated with low-dose STZ (Sigma-Aldrich, St Louis, MO, USA). In the next step, the low-dose STZ, that is, 30 mg/kg dissolved in 0.1 M sodium-citrate buffer at a pH of 4.4, was injected to the animals intraperitoneally. The level of blood glucose was also tested after the first week with a blood glucose meter. Accordingly, the animals with blood glucose levels >16.7 mmol/l were injected with STZ (30 mg/kg) for the second time. After that, the sham group was injected with citrate buffer (as a vehicle) (0.25 ml/kg). It is notable that the mentioned diets were maintained at the post-injection stage. After four weeks, each rat with the blood glucose concentration <16.7 mmol/l was regarded to be diabetic and thus was chosen for additional investigations.[25]

Subsequently, HFD/STZ-induced T2D old male Wistar rats were fed with the high-fat diet (55% fats, 31% carbohydrates, and 14% protein). The sham animals also received a standard diet (10% fats, 75% carbohydrates, and 15% protein) throughout the study. The HFD plus UA (250 mg UA per kg of body weight rat/day) was further prepared by mixing 500 mg of UA per kg of HFD (0.5% UA plus HFD, Royan Company, Isfahan. Iran.).[26] It should be noted that the high-fat diet plus UA was prepared at the three-day interval to avoid the oxidation of fat or other compounds (Knowledge-Based Company, Healthy-Aging Supplement 9870, Tehran, Iran). The daily mean amount of the food intake was calculated as a difference between the amount of the remained food and the total one provided, divided by the number of days and rats in cages. Since the energetic values between the diets differed, the use of food in grams was converted into the caloric intake[27], and finally, weekly body weight for each rat was measured during the investigation.[28]

Determination of velocity at maximal oxygen uptake ($vVO_{2\ max}$)

To evaluate $vVO_{2\ max}$, a ten three-min phase running test on a rodent treadmill was used. According to Leandro *et al*.,[29] initial running speed test was equal to 0.3 km/h and then speed elevated by 0.3 km h^{-1} per 3 min (the slope was

equal to 0%). Therefore, in case of the rats' inability to continue the running in all phases of the experiment, the speed at that phase was considered as $vVO_{2\ max}$.

Determination of maximum voluntary carrying capacity (MVCC)

In this step, the animals were let to be familiarized with a vertical climbing model (110 cm, 2-cm grid, 85° incline) without overloading.[30] Following a 72-h familiarization, all the animals were also tested to assess the respective MVCC. However, all of them carried a load of 75% of the body weight to the top of the ladder (namely, a house chamber) for the first climb. Then, the rats were allowed to rest for 120 s. After that, some weight increments of 30 g were added till the load did not let the rats climb the whole length of the ladder. It is noteworthy that the given process was iterated, so that the animals were not voluntarily capable of climbing the entire length of the ladder on three subsequent efforts. Finally, the MVCC was viewed as the successfully greatest load over the whole length of the ladder.[30]

Resistance training protocol

Initially, the rats became familiarized with the apparatus by climbing it without the weights attached to their tails. Moreover, the weights were then fastened to the animals' tails. In crucial circumstances, a physical stimulus with finger pinching on the rats' tails was further employed as a stimulus for initiating the climbing movements. Upon the arrival of the animals in a housing chamber, they were let to rest for two min. Then, the procedure was iterated till the animals could voluntarily climb for three consecutive attempts.[31] The resistance training group also performed the ladder resistance training at 60% of the MVCC, with 14, 16, 18, 20 climbs in each session, respectively, in weeks 1–2, 3–4, 5–6, and 7–8, with a one-min rest between each two trials, five days a week.[32] Finally, the group with diabetes was restricted to do any physical exercise training beyond the normal cage activities.

Endurance training protocol

Within the eight-week training, the animals were exposed to endurance training programs. Then, the training intensity was calculated by $vVO_{2\ max}$. At the beginning of the endurance training, the animals were trained at 40–50% of $vVO_{2\ max}$ for five min and 0% incline for warm-up. The endurance training protocol consisted of repeated bouts of high- and low-intensity training, two min of running with 60% $vVO_{2\ max}$ in the course of the first week, 65% $vVO_{2\ max}$ during the second week, 70% $vVO_{2\ max}$ in the course of the third week, and finally 75% $vVO_{2\ max}$ during the fourth week to the completion of the training time. Moreover, low-intensity bouts involved two-min running with 40% $vVO_{2\ max}$ from the first week to the end of the third week and 30% $vVO_{2\ max}$ from the onset of the fourth week to the completion of the eighth week. At the end, the number of the high-intensity interval bouts increased from two to eight reps from the first to the end of the eighth week.[33]

Hippocampus tissue collection

The rats were weighed and anesthetized through intraperitoneal administration of a mixture of 90 mg/kg ketamine and 10 mg/kg xylazine. To avoid the acute effects of the last training/supplementation sessions, the animals were then sacrificed 48 h following the final resistance/endurance training session. The hippocampus tissues were then gently dissected out and immediately immersed in liquid nitrogen.

Western blotting

According to the research design, Western blotting was run on the homogenates of the hippocampus tissues. Briefly, a pestle was employed to powder about 50 mg of the hippocampus tissue piece in liquid nitrogen and then lysed with a 1 mL of phosphate buffered saline (PBS). Moreover, this buffer was complemented with a protease inhibitor cocktail consisting of pepstatin, leupeptin, aprotinin, antipain, as well as chymostatin (5 μg/ml each) and rotated for 20 min at 4°C. In addition, they were centrifuged at 12,000×g at a temperature of 4°C for 10 min. Hence, this supernatant was gathered and kept at −80°C. The total protein content of the tissue extract was correspondingly determined by the Bradford method (Bio-Rad Laboratories, CA, USA) and spectrophotometric measurements (Jenway™ 6305 UV/Visible Spectrophotometer, Bibby Scientific Ltd., UK). The proteins were then isolated through the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique (10 μg protein loaded in the wells) and transported electrophoretically over the polyvinylidene fluoride or polyvinylidene difluoride (PVDF) membranes. It should be noted that the non-specific binding was further obstructed by a 2-h incubation of the membrane in 5% (w/v) non-fat dry milk in the Tris-buffered saline (TBS) at a power of hydrogen (pH) of 7.5. After that, the incubation of the blots was done for 2 h at the room temperature or overnight at a temperature of 4°C with the primary antibodies; that is, anti-BDNF, IGF-1 as well as β-actin (Santa Cruz, USA) 1:500, diluted in an antibody buffer consisting of 1% (w/v) non-fat dry-milk in TBS and polysorbate 20 (Tween 20) (0.05% (v/v) in the TBS). Afterward, they were washed three times with TBS-T, and consequently, incubation was fulfilled for 1 h with a secondary antibody, that is, goat anti-rabbit (IgG) (Santa Cruz, USA) 1:5000, in the antibody buffer. Next, the blots were developed to visualize by means of the enhanced chemiluminescence (ECL) detection kit (Pierce, Rockford, IL, USA). Finally, antiβ-actin was employed as one of the loading controls. The band intensity on immunoblots was further quantified via densitometry using the Image Studio Lite software.

Blood Glucose and Insulin measurement

In this step, fasting blood glucose (FBG) in the tail vein blood sample was measured at the beginning of the experiment through a blood glucose meter to ensure that the animals had euglycemia. Following the HFD/STZ-induced T2D, blood glucose level was measured to assess the initiation of hyperglycemia (FBG 200 mg/dl). Moreover, the enzyme-linked immunosorbent assay (ELISA) (Mecodia, Winston-Salem Inc., NC, USA) was utilized to measure the plasma insulin levels in the rats that had been fasted for four h.

Statistical analysis

According to the research design, the statistical analyses were completed using the SPSS Statistics software (ver. 21). Then, two-way analysis of variance (ANOVA) with training conditions in three levels (namely, resistance, endurance, and no exercise training) and supplementation in two levels (UA and no supplementation) were applied to reflect on the impact of the training as well as supplementation on modifications in the serum and gene expression outcomes. As well, the two-way ANOVA with group status in six levels (namely, D, DE, DR, DU, DEU, and DRU) and time with three to eight levels (that is, three levels for vVo_2 and MVCC [i.e., weeks one, four, and eight], and eight levels for body weight [namely, weeks one, two, three, four, five, six, seven, and eight]) was employed to determine the impact of group and time status on the alterations in descriptive outcomes. It should be noted that the sham group (C) data were not included in the statistical analysis. Furthermore, Tukey's test was employed for each analysis, which required a post hoc test. To examine the correlation between the parameters, the Pearson correlation coefficient was consequently practiced. The level of statistical significance was estimated to be $P < 0.05$.

Results

Blood glucose and weight changes in rats during the study in each group

The two-way ANOVA results did not show any remarkable group main effect ($P = 0.151$), time main effect ($P = 0.091$), as well as group by time interaction effect ($P = 0.998$) on the body weight following the eight weeks of interventions.

No significant group main effect ($P = 0.071$) and group by time interaction effect ($P = 0.307$) were also observed, but a considerable time main effect ($P = 0.017$) was reported on the glucose levels after the completion of the eight-week interventions. The post hoc comparisons for the time main effect correspondingly showed that the glucose levels had significantly decreased in the fourth ($P = 0.045$) and sixth weeks ($P = 0.042$) compared with the first one.

MVCC and $vVO_{2\ max}$ changes in study groups

A significant group main effect ($P = 0.001$) and a time main effect ($P = 0.001$), but no group by time interaction effect ($P = 0.178$) was observed for the MVCC. The post hoc comparisons for the group main effect also revealed that the MVCC had remarkably enhanced in the DR ($P = 0.010$) and the DRU ($P = 0.001$) groups compared with that in the D one, in the DRU ($P = 0.001$) as well as the DR groups ($P = 0.015$) in comparison with the UA one, as well as in the DRU group ($P = 0.002$) as compared with the DE one. Moreover, the post hoc comparisons for the time main effect demonstrated that the MVCC had been significantly boosted in the posttest in comparison with that in the mid-test ($P = 0.001$) and the pretest ($P = 0.004$).

As well, the $vVo_{2\ max}$ showed a significant main group effect ($P = 0.001$), time main effect ($P = 0.001$), as well as group by time interaction effect ($P = 0.001$). The post hoc comparisons for the group main effect also revealed that the $vVo_{2\ max}$ had significantly increased in the DEU, DE, DRU, and DU groups compared with the D one. This was similarly evident in the DEU, DE, and DRU groups compared with the DR one; as well as in the DEU and DE groups in comparison with the DRU and DU ones ($P < 0.05$).

The post hoc comparisons for the time main effect correspondingly revealed that the $vVo_{2\ max}$ had remarkably elevated in the mid-test ($P = 0.019$) and the posttest ($P = 0.001$) as compared with that in the pretest. The post hoc comparisons for the group by time interaction effect additionally established that the $vVo_{2\ max}$ had significantly increased in the DEU and DE groups compared with that in the D, DR, DRU, and DU ones; as well, in the DRU group in comparison with the D and DR ones in the mid-test. Moreover, a significant growth in the $vVo2_{\ max}$ in the posttest was observed in the DEU and DE groups compared with that in the D, DR, DRU, and DU ones, and in the DRU group compared with that in the D one in the mid-test ($P < 0.05$) [Figure 1].

Serum glucose and insulin changes in study groups

The two-way ANOVA results demonstrated no remarkable training main effect ($P = 0.465$), supplement main effect ($P = 0.976$), as well as training by supplement interaction effect ($P = 0.534$) on the glucose levels. Similarly, there were no significant training main effect ($P = 0.163$), supplement main effect ($P = 0.138$), and training by supplement interaction effect ($P = 0.327$) on the insulin [Figure 2].

Expression of BDNF, IGF-1 changes in hippocampus tissue in study groups

BDNF/B-actin indicated significant exercise main effect ($P = 0.001$) and supplement main effect ($P = 0.001$), but not exercise by supplement

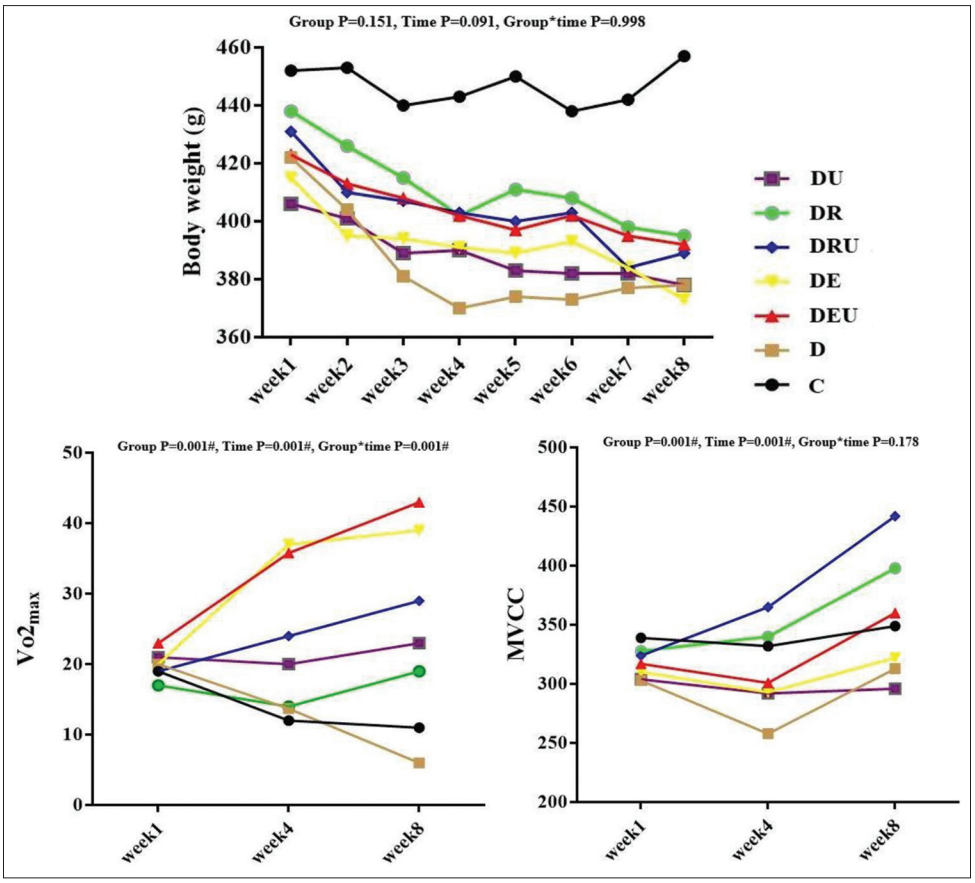

Figure 1: Body weight, MVCC, and VO2 $_{max}$ changes following eight-week exercise training and ursolic acid. DU: Diabetic + Ursolic Acid; DR: Diabetic + Resistance Training; DRU: Diabetic + Resistance Training + Ursolic Acid; DE: Diabetic + Endurance Training; DEU: Diabetic + Endurance Training + Ursolic Acid; D: Diabetic; C: Control

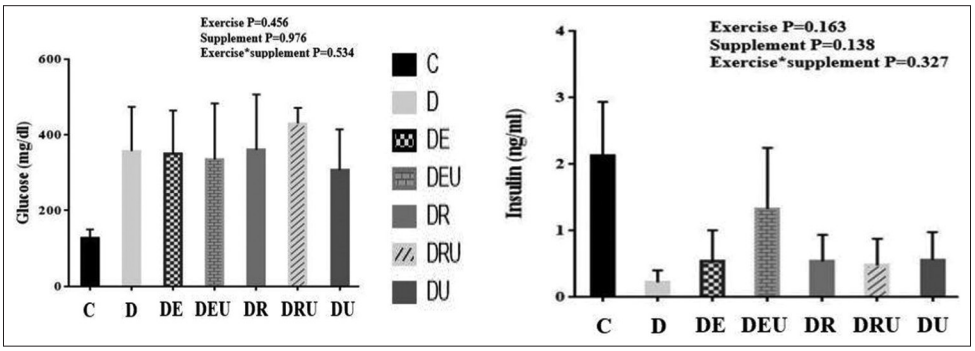

Figure 2: Insulin changes following eight-week exercise training and ursolic acid. DU: Diabetic + Ursolic Acid; DR: Diabetic + Resistance Training; DRU: Diabetic + Resistance Training + Ursolic Acid; DE: Diabetic + Endurance Training; DEU: Diabetic + Endurance Training + Ursolic Acid; D: Diabetic; C: Control

interaction effect (P = 0.191). Post hoc comparison of exercise effect showed that BDNF/B-actin significantly increased in endurance training condition compared to resistance and none training condition (*both*, P = 0.001). Also, post hoc comparison of supplement effect showed that BDNF/B-actin significantly increased in ursolic acid condition (P = 0.001) compared to none supplementation condition. IGF-1/B-actin indicated significant supplement main effect (P = 0.020), but not exercise main effect (P = 0.194) and exercise by supplement interaction effect (P = 0.448). Post hoc comparison of supplement effect showed that IGF-1/B-actin significantly increased in ursolic acid condition (P = 0.020) compared to none supplementation condition. [Figure 3].

Behavioral test of memory and learning

The two-factor ANOVA test for time-1 in shuttle box test indicated no significant exercise main effect (P = 0.677), supplement main effect (P = 0.139), and exercise by supplement interaction effect (P = 0.758). Also, time-2 in shuttle box test indicated no significant exercise main effect (P = 0.237), supplement main

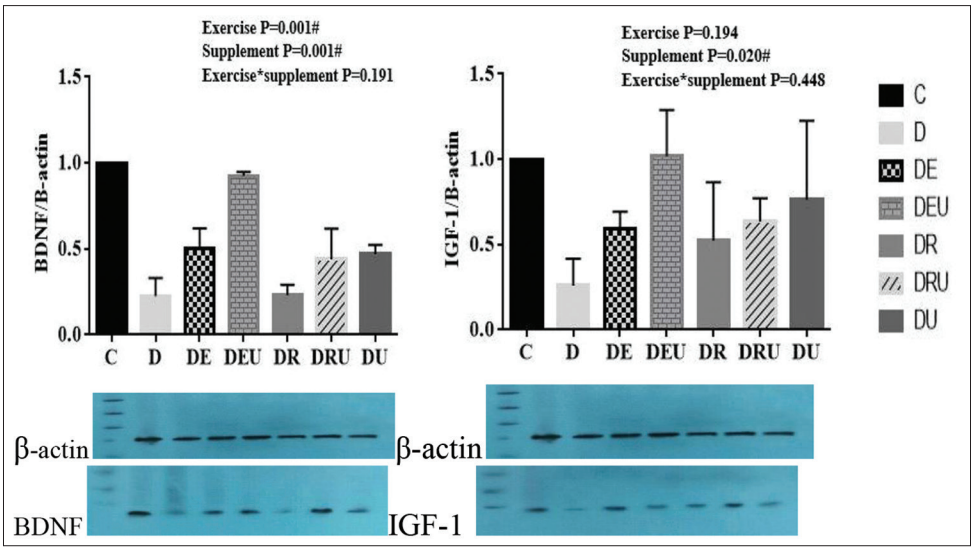

Figure 3: BDNF and IGF-1 gene expression changes following eight-week exercise training and ursolic acid. DU: Diabetic + Ursolic Acid; DR: Diabetic + Resistance Training; DRU: Diabetic + Resistance Training + Ursolic Acid; DE: Diabetic + Endurance Training; DEU: Diabetic + Endurance Training + Ursolic Acid; D: Diabetic; C: Control

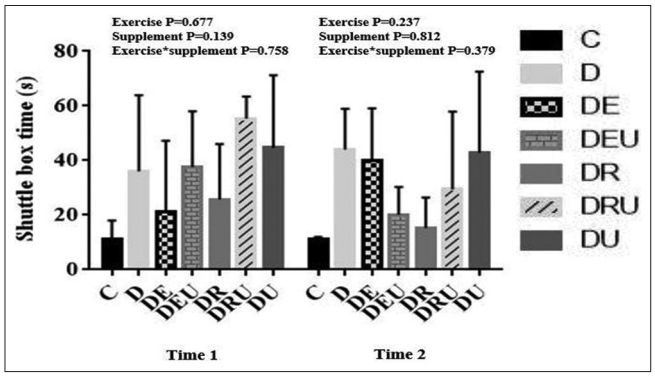

Figure 4: Shuttle box time following eight-week exercise training and ursolic acid. DU: Diabetic + Ursolic Acid; DR: Diabetic + Resistance Training; DRU: Diabetic + Resistance Training + Ursolic Acid; DE: Diabetic + Endurance Training; DEU: Diabetic + Endurance Training + Ursolic Acid; D: Diabetic; C: Control. Diabetic + Endurance Training; DEU: Diabetic + Endurance Training + Ursolic Acid; D: Diabetic; C: Control

effect (P = 0.812), and exercise by supplement interaction effect (P = 0.379) [Figure 4].

Discussion

Type 2 DM is associated with dysregulation of the insulin signaling pathway, reduction of neuronal proliferation and synaptic plasticity, and downregulation of melatonin receptors in rat hippocampus. These insulin-induced molecular dysregulations result in learning and memory deficits, as well as general impairment of cognitive functions.[34]

Our results showed differences in glucose and insulin index and changes were observed in BDNF and IGF-1 amounts in hippocampus in HFD-STZ diabetic aged rats which will be examined.

Other studies in this field also suggested an association of diabetes and cognitive dysfunction and revealed that diabetes is an independent risk factor for neurological disorders.[35]

Ursolic acid a lipophilic pentacyclic triterpenoid present as waxy coat on apples and many herbs and it is known to possess a number of biological activities such as anti-inflammatory, antidiabetic, antioxidant, anticancer, and hepatoprotective. Some studies have reported improvement in glucose tolerance by UA treatment.[11] The mechanisms underlying these effects of UA supplementation in HFD-induced obese rats are due to an increase in Akt phosphorylation and an improvement in glucose uptake by skeletal muscles. Therefore, these findings highlight the importance of UA in the treatment of obesity and diabetes.[36]

Regular physical exercise induced a significant increase in the pancreatic islet size and insulin secretion.[34]

Although both aerobic exercise and resistance training were effective in improving insulin sensitivity, and that combining the two was the most efficient strategy. The intensity and duration of exercise are also proposed to have a dose effect on both the mechanisms and positive cognitive outcomes.[37]

BDNF in hippocampus tissue indicated significant exercise main effect and supplement main effect, but not exercise by supplement interaction effect. Post hoc comparison of exercise effect showed that BDNF significantly increased in endurance training condition compared to resistance and none training condition. Also, post hoc comparison of supplement effect showed that BDNF significantly increased in ursolic acid condition compared to none supplementation condition.

BDNF is involved in neurogenesis and memory function, including neuronal connectivity, synaptic development and

plasticity; in addition, it enhances the insulin sensitivity in patients with T2D.[38] However, increasing/growing evidence demonstrated that BDNF protein was decreased in the animal and patients with AD and T2D and treadmill exercise induced up-regulation of the BDNF in the cortex of Tg mice and may also be involved in the neuroprotective and insulin sensitivity.[38,39]

IGF-1 indicated significant supplement main effect, but not exercise main effect and exercise by supplement interaction effect. Post hoc comparison of supplement effect showed that IGF-1 significantly increased in ursolic acid condition compared to none supplementation condition.

Physical activity induces neurotransmitters and growth factors (BDNF, IGF-1) increase circulating testosterone levels and decrease insulin resistance. Increased cerebral blood flow in response to greater physical activity may also have a mechanistic role in this association.[40] Furthermore, IGF-I increases levels of the BDNF receptor (TrkB), which in turn increases levels of BDNF signaling.[41]

In rats, both aerobic and resistance training improved learning and spatial memory after eight weeks, but did so with differing signaling pathways.[42] Aerobic exercise also activates the PI3K/Akt/mTOR and AMPK/Sirt1 signaling pathways and inhibits the NFκB/NLRP3/IL-1β signaling pathway in the hippocampus of diabetic rats. Therefore, modulating the PI3K/Akt/mTOR, AMPK/Sirt1, and NFκB/NLRP3/IL-1β signaling pathways is probably the mechanism of aerobic exercise upregulating the expression of hippocampal synaptic plasticity-associated proteins in diabetic rats.[43] What has been mentioned recently is that aerobic and multimodal training either increased or maintained BDNF concentrations and resistance training either increased or maintained IGF-1 concentrations.[44]

Despite the positive results of exercise and supplementation with cognitive disorders caused by diabetes, we did not achieve these results by performing the shuttle box test.[45]

Therefore, we investigate the effect of endurance/resistance training and ursolic acid supplement on hippocampus tissue biomarkers of type 3 diabetes (BDNF and IGF-1) in diabetic aged rats by HFD and STZ.

Although this study tried to minimize the limitations, however, there were some limitations in the present study. The measurement of other neurotrophic factors as well as neurogenesis indices could give us a more complete view along with the indices present in the study. Also, if different intensities of exercise training and different doses of ursolic acid supplement were used, more comprehensive information could be obtained, but this was not possible due to the multiplicity of groups.

Conclusions

Overall, the results of this study showed that endurance/resistance training and ursolic acid supplement can reduce cognitive impairments caused by diabetes and it affects type 3 diabetes biomarkers. Understandably, this study showed that exercise and ursolic acid supplementation can have protective effects against diabetes-related disorders that can be used as an effective and uncomplicated method to be used to reduce brain complications caused by diabetes referred to as type 3 diabetes in recent studies.

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Ethics approval and consent to participate

All animal procedures were approved by Animal Ethics Committee (Shahrekord University, Iran) and complied with the Guide for Care and Use of Laboratory Animals.

Availability of data and materials

All the data generated or analyzed during the present study were included in this paper.

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Conflicts of interest

There are no conflicts of interest.

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