



Comparison of High- and Moderate-Intensity Aerobic Training on Cerebellar Autophagy in Aged Rats With Induced Diabetes

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ABSTRACT

Aging and diabetes are associated with impaired autophagic function, which can lead to the accumulation of cellular damage, accelerated cellular senescence, and increased vulnerability of neural tissues, particularly the cerebellum. Aerobic exercise has been proposed as a non-pharmacological strategy for improving autophagy and attenuating the adverse consequences of aging and diabetes; however, the comparative effects of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) on autophagy-related markers in the cerebellum of aged diabetic rats have not been fully elucidated. The aim of this study was to compare the effects of MICT and HIIT on markers of autophagy and cellular senescence in the cerebellum of aged rats with diabetes. In this experimental study, rats were initially maintained on either a standard diet or a high-fat, high-fructose diet. At 6 months of age, the young control groups were sampled. After the animals reached 18 months of age, they were assigned to four groups: aged healthy control, aged diabetic control, aged diabetic + MICT, and aged diabetic + HIIT. The exercise programs were conducted for 3 months. Diabetes induction was confirmed by assessment of blood glucose and insulin resistance. Forty-eight hours after the final exercise session, cerebellar tissue was collected, and the expression levels of LC3-I, LC3-II, and Beclin-1 were measured using Western blotting. The LC3-II/LC3-I ratio was also calculated as an index of autophagic flux, and senescence-associated β -galactosidase (SA- β -gal) activity was assessed using ELISA. The results showed that aging combined with diabetes increased LC3-I expression, decreased the LC3-II/LC3-I ratio, and reduced Beclin-1 expression in the cerebellum, indicating impaired autophagy. SA- β -gal activity was also significantly increased in the aged diabetic group. Both exercise protocols partially reversed these alterations; however, MICT was more effective than HIIT in increasing the LC3-II/LC3-I ratio, improving Beclin-1 expression, and reducing SA- β -gal activity. Overall, aging and diabetes impair autophagy and increase cellular senescence in the cerebellum, and both forms of aerobic exercise exert protective effects; nevertheless, MICT appears to be more effective than HIIT.

Keywords: autophagy, LC3, Beclin-1, SA- β -gal, moderate-intensity continuous training, high-intensity interval training, diabetes, aging, cerebellum.

1. Introduction

Aging is a complex biological process characterized by the progressive deterioration of cellular homeostasis, reduced adaptive capacity, accumulation of molecular damage, and increasing vulnerability of tissues to metabolic and neurodegenerative disorders. Among organ systems, the central nervous system is particularly susceptible to age-related alterations because neurons are highly differentiated, long-lived cells with substantial energetic demands and limited regenerative capacity. Structural and functional changes in the aging brain include neuronal loss in selected regions, impaired synaptic plasticity, mitochondrial dysfunction, altered protein quality control, oxidative stress, and reductions in the efficiency of cellular clearance mechanisms (1, 2). These changes may compromise neural resilience and contribute to progressive deterioration of motor, cognitive, and integrative functions. Although aging affects multiple brain regions, the cerebellum is increasingly recognized as an important target of age-related structural, metabolic, and molecular alterations rather than merely a region involved in the coordination of movement (3).

The cerebellum plays a central role in motor coordination, postural control, balance, motor learning, and temporal processing, while accumulating evidence also implicates cerebellar circuits in cognitive and affective regulation. With advancing age, the cerebellum undergoes changes in neuronal morphology, synaptic organization, vascular integrity, mitochondrial function, and intracellular proteostasis, potentially reducing its ability to respond effectively to metabolic and cellular stress (3). Cerebellar aging may therefore have broad functional consequences, particularly when it occurs in the presence of metabolic disorders that further disturb cellular energy balance. Age-related changes in the brain do not arise from a single pathological pathway; rather, they reflect the interaction of mitochondrial damage, impaired lysosomal clearance, altered inflammatory signaling, oxidative injury, and reduced turnover of damaged cellular components (1, 4). Among these interconnected mechanisms, autophagy is considered a key determinant of neural homeostasis and healthy aging.

Autophagy is a highly conserved intracellular degradation and recycling process through which damaged proteins, dysfunctional organelles, and other cytoplasmic

components are sequestered and delivered to lysosomes for degradation. This process is essential for maintaining cellular quality control, regulating energy availability during stress, and preventing the accumulation of potentially toxic intracellular material. In neurons, efficient autophagy is particularly important because the limited proliferative potential of these cells makes intracellular repair and recycling mechanisms critical for long-term survival. Experimental and comparative evidence indicates that preserved autophagic activity is strongly associated with longevity, whereas disruption of autophagy accelerates age-related functional decline and increases susceptibility to degenerative processes (5). Autophagy therefore functions not only as a housekeeping mechanism but also as an important regulator of cellular survival, metabolic adaptation, and organismal aging.

The autophagic pathway involves several sequential stages, including initiation, autophagosome formation, cargo sequestration, fusion with lysosomes, and degradation of the engulfed material. Two frequently investigated molecular markers in this pathway are microtubule-associated protein 1 light chain 3 (LC3) and Beclin-1. During autophagosome formation, the cytosolic form LC3-I is lipidated to form membrane-associated LC3-II, which is incorporated into autophagosomal membranes. Changes in LC3-I and LC3-II, as well as the LC3-II/LC3-I ratio, are therefore commonly used to characterize changes in autophagy-related processes. Beclin-1, in turn, is a key regulator of autophagosome nucleation and participates in the early stages of autophagy initiation. Dysregulation of these proteins may reflect reduced autophagosome formation, impaired turnover, or disturbance of autophagic homeostasis (2, 5). In aging neural tissue, such disturbances may contribute to the accumulation of defective proteins and organelles that would otherwise be removed through lysosomal degradation.

Age-related impairment of autophagy appears to be especially important in the brain, where defective clearance of damaged mitochondria and aggregated proteins can progressively compromise neuronal function. Autophagy and the selective degradation of damaged mitochondria, known as mitophagy, are closely linked to neuronal energy metabolism, redox balance, and resistance to stress. Compromised autophagy and mitophagy have been documented in brain aging and are increasingly considered

mechanistic contributors to neurodegenerative processes (4). Failure to eliminate damaged mitochondria may increase reactive oxygen species production, impair ATP synthesis, and intensify cellular stress, while inadequate removal of misfolded or aggregated proteins further disrupts intracellular homeostasis. This relationship suggests that interventions capable of preserving or restoring autophagic and lysosomal function may have substantial relevance for protecting the aging brain.

Diabetes represents an additional and clinically important factor that may accelerate age-related neural deterioration. Type 2 diabetes mellitus is characterized by chronic hyperglycemia, insulin resistance, dysregulated lipid metabolism, and systemic metabolic stress, all of which can negatively affect the central nervous system. Diabetes in older adults has been associated with cognitive dysfunction, cerebrovascular abnormalities, brain structural changes, and increased susceptibility to neurodegenerative disorders (6). More recent evidence suggests that individuals with diabetes may exhibit accelerated brain aging and that poor glycemic control is associated with more pronounced brain-aging phenotypes and adverse long-term outcomes (7). These findings support the concept that diabetes may amplify biological aging in neural tissue through mechanisms involving glycemic toxicity, vascular dysfunction, inflammation, oxidative stress, and impaired cellular maintenance.

The combination of aging and diabetes is therefore likely to produce a particularly unfavorable intracellular environment. Persistent hyperglycemia and insulin resistance can impair mitochondrial function, promote oxidative damage, alter nutrient-sensing pathways, and disrupt autophagic regulation. When superimposed on the natural age-related decline in proteostasis and lysosomal efficiency, these alterations may intensify the accumulation of dysfunctional cellular components. Experimental diet-induced models have been widely used to reproduce key metabolic characteristics of human type 2 diabetes. Long-term consumption of high-fat and high-fructose diets in rats can induce severe obesity, insulin resistance, ectopic lipid accumulation, and marked disturbances in glucose metabolism (8). Similarly, combined high-fructose and high-fat feeding has been shown to produce metabolic abnormalities relevant to diabetes risk and its systemic

complications (9). Such models are therefore useful for examining the interaction between prolonged metabolic dysfunction and aging at the tissue and molecular levels.

Cellular senescence is another major process linking aging, diabetes, and tissue dysfunction. Senescent cells exhibit a state of stable proliferative arrest accompanied by profound changes in metabolic and secretory activity. The accumulation of senescent cells can promote tissue inflammation, impair regenerative signaling, and contribute to age-related functional decline. Senescence-associated β -galactosidase (SA- β -gal) activity is widely used as a biochemical indicator of cellular senescence and provides a complementary perspective to molecular indices of autophagy. Because impaired autophagy may facilitate the persistence of damaged organelles and proteins, while cellular senescence itself may further disrupt proteostatic regulation, these two processes can interact to accelerate tissue deterioration (2, 5). Evaluating autophagy-related proteins together with a senescence marker may therefore provide a more comprehensive assessment of cerebellar cellular status in aging and diabetes.

Among non-pharmacological interventions, regular physical exercise is considered one of the most effective strategies for improving metabolic health, maintaining functional capacity, and attenuating age-associated physiological decline. Aerobic exercise can improve insulin sensitivity, glucose regulation, mitochondrial function, vascular health, inflammatory status, and overall metabolic efficiency. Evidence from human studies further supports the role of structured physical activity in modifying metabolic and inflammatory outcomes in individuals with obesity and related cardiometabolic risk factors (10, 11). These systemic benefits are particularly relevant to diabetes and aging because chronic metabolic dysfunction and low-grade inflammation can negatively influence both peripheral tissues and the central nervous system. However, the biological effects of exercise are not limited to systemic metabolism; exercise can also activate intracellular signaling pathways involved in cellular repair, organelle turnover, and lysosomal function.

One potential mechanism by which exercise may protect neural tissue is the activation of autophagy and lysosomal biogenesis. Experimental evidence indicates that exercise can stimulate lysosomal function in the brain through

pathways involving AMP-activated protein kinase (AMPK), sirtuin 1 (SIRT1), and transcription factor EB (TFEB), thereby facilitating cellular clearance and adaptive remodeling (12). Activation of these nutrient- and energy-sensing pathways is biologically important because AMPK responds to cellular energetic stress, SIRT1 integrates metabolic and stress signals, and TFEB regulates genes involved in lysosomal biogenesis and autophagy. Through these mechanisms, aerobic exercise may enhance the removal of damaged proteins and organelles and help maintain neuronal homeostasis under conditions of aging and metabolic disease. This provides a strong mechanistic rationale for examining exercise-induced changes in cerebellar autophagy.

Nevertheless, the magnitude and direction of exercise-induced autophagic adaptations may depend strongly on exercise intensity. Moderate-intensity continuous training is characterized by sustained aerobic activity performed at a submaximal workload and is widely used to improve cardiovascular and metabolic function. High-intensity interval training, in contrast, consists of repeated bouts of relatively intense activity separated by periods of lower-intensity exercise or recovery. Both modalities can produce substantial cardiometabolic adaptations, but they impose different patterns of metabolic stress, energy demand, lactate production, mitochondrial challenge, and recovery. In animal models of low aerobic capacity and metabolic syndrome, both interval and continuous aerobic exercise have been shown to improve several metabolic and cardiovascular outcomes, although the magnitude of adaptation can vary between protocols (13). Such differences raise the possibility that MICT and HIIT may also differentially affect autophagic regulation in neural tissue.

Accurate comparison of exercise protocols requires appropriate quantification of exercise intensity. In rodent studies, maximal running capacity and maximal oxygen consumption are commonly used to standardize relative exercise loads and minimize variation caused by differences in baseline fitness. Experimental work in male Wistar rats has demonstrated the feasibility of characterizing maximal oxygen consumption and quantifying exercise intensity through graded treadmill testing (14). Individualized intensity prescription is particularly important in aged and

metabolically impaired animals because identical absolute treadmill speeds may represent substantially different physiological loads across individuals. Therefore, defining MICT and HIIT relative to maximal exercise capacity improves the methodological validity of direct comparisons between exercise modalities.

Although HIIT has received considerable attention because of its capacity to produce robust metabolic adaptations within relatively short exercise durations, its effects on the aging brain may not always be uniformly beneficial. High-intensity exercise imposes substantial energetic and mitochondrial stress, and the response may vary according to age, disease status, tissue type, and training volume. In middle-aged mice, HIIT has been associated with dysregulation of mitochondrial respiratory complexes and mitophagy within the hippocampus under certain experimental conditions (15). This finding suggests that a higher exercise intensity does not necessarily produce superior neural adaptations and that excessively demanding workloads may disturb mitochondrial quality-control mechanisms in susceptible tissues.

Conversely, other findings indicate that HIIT can exert beneficial effects on neural pathology in metabolic disease when appropriately prescribed. In rats with type 2 diabetes, HIIT has been reported to reduce tau and β -amyloid accumulation through improvements in lactate-dependent mitophagy, suggesting that intense interval exercise may enhance mitochondrial quality control and attenuate protein-related neuropathological changes (16). These apparently differing observations emphasize the context-dependent nature of exercise-induced autophagic and mitophagic responses. Age, diabetic status, brain region, protocol duration, exercise dose, and baseline mitochondrial function may all determine whether a given training stimulus produces adaptive or maladaptive cellular responses.

This issue is particularly relevant to the cerebellum because relatively less attention has been devoted to this brain region compared with the hippocampus and cerebral cortex. Contemporary evidence increasingly indicates that cerebellar aging involves molecular and cellular disturbances that may contribute to motor, cognitive, and neurodegenerative outcomes (3). Yet the extent to which prolonged diabetes aggravates cerebellar autophagic impairment and whether exercise intensity modifies this

response remain insufficiently characterized. Given the central role of autophagy in neuronal proteostasis and longevity, clarifying these relationships may improve understanding of how exercise preserves neural integrity during aging and metabolic disease.

The interaction between exercise intensity and cellular senescence is likewise of interest. Exercise-induced improvements in glycemic control, body composition, inflammatory status, and mitochondrial function may reduce cellular stress and indirectly attenuate senescence-associated changes. Physical activity interventions have demonstrated favorable effects on metabolic and inflammatory variables in populations with obesity, supporting the broader premise that exercise can modify biological pathways linked to chronic metabolic stress (10, 11). However, whether moderate continuous exercise and high-intensity interval exercise differ in their ability to reduce senescence-related activity in aged diabetic cerebellar tissue remains unclear. Simultaneous assessment of SA- β -gal and autophagy-related proteins may therefore help determine whether exercise-induced changes in cellular quality control are accompanied by changes in cellular aging.

Taken together, available evidence supports a close association among aging, diabetes, autophagic dysfunction, mitochondrial impairment, and neural vulnerability. Aging reduces the efficiency of intracellular quality-control systems, while diabetes imposes additional metabolic and oxidative stress that may accelerate brain aging (6, 7). Autophagy appears to be a central protective mechanism against these processes, and its preservation has been linked to longevity and neuronal homeostasis (4, 5). Exercise is capable of activating molecular pathways involved in lysosomal function and organelle turnover (12), yet different exercise intensities may produce distinct effects on mitochondrial and autophagic regulation (13, 15, 16). Accordingly, direct comparison of MICT and HIIT in an aged diabetic model is necessary to determine whether the magnitude of exercise intensity influences the restoration of autophagy and attenuation of cellular senescence in the cerebellum.

Therefore, the aim of the present study was to compare the effects of moderate-intensity continuous training and high-intensity interval training on LC3-I, LC3-II, Beclin-1,

the LC3-II/LC3-I ratio, and SA- β -gal activity in the cerebellum of aged rats with induced diabetes.

2. Methods and Materials

2.1. Study Design and Experimental Animals

This experimental study employed a posttest-only control-group design to investigate and compare the effects of two aerobic exercise modalities, namely moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT), on autophagy-related markers in the cerebellar tissue of aged diabetic rats. The study was designed to evaluate the effects of aging, diabetes, and exercise intervention simultaneously.

The study population consisted of male Wistar rats with an initial age of approximately 10 weeks and a mean initial body weight of 200 ± 20 g. The animals were obtained from the Laboratory Animal Research and Breeding Center of Ahvaz Jundishapur University of Medical Sciences. After transfer to the laboratory, the animals were maintained under standard conditions for 2 weeks to minimize transportation-related stress and allow adaptation to the new environment. During this period, all animals were exposed to identical environmental conditions, and no experimental intervention was performed.

The animals were housed three per cage in standard transparent autoclavable polycarbonate cages. Sterile wood shavings were used as bedding and replaced regularly to maintain environmental hygiene. The housing temperature was maintained at $22 \pm 2^\circ\text{C}$, relative humidity at 40%–60%, and the light–dark cycle at 12 hr of light and 12 hr of darkness. Appropriate ventilation was maintained continuously to prevent the accumulation of ammonia and other environmental contaminants. Drinking water and food were provided *ad libitum* throughout the study.

All stages of the study were conducted in accordance with national institutional guidelines for the care of laboratory animals and the principles of the Helsinki Protocol (2006). The study was also approved by the Ethics and Research Committee of Islamic Azad University, Ahvaz Branch, under ethics code IR.IAU.AHVAZ.REC.1404.003 on April 5, 2025. Every effort was made to minimize animal stress and pain throughout the study period.

2.2. Experimental Design and Animal Grouping

Following the adaptation period, the animals were randomly assigned to two main groups: a standard-diet group and a high-fat plus fructose diet group. The purpose of this stage was to establish an experimental model of metabolic abnormalities resembling type 2 diabetes and to investigate its interaction with the aging process in cerebellar tissue.

In the first stage of the study, when the animals reached 6 months of age, three rats from the standard-diet group and three rats from the high-fat/high-fructose diet group were selected as the young control groups. To investigate the initial effects of the dietary regimens on metabolic status and the study variables, these animals were anesthetized and cerebellar tissue samples were collected without any exercise intervention.

The remaining animals were maintained until 18 months of age to establish the aging model. Upon entering the aged stage, the animals were randomly allocated to four experimental groups: aged healthy control, aged diabetic control, aged diabetic rats undergoing moderate-intensity continuous training (MICT), and aged diabetic rats undergoing high-intensity interval training (HIIT). In addition to continuing the high-fat, high-fructose diet, both exercise groups completed their respective training programs for 3 months, until the animals reached 21 months of age.

2.3. Diet and Diabetes Induction

The standard diet consisted of a balanced mixture of protein, carbohydrate, and fat specifically formulated for the maintenance of laboratory animals. Approximately 20% of the total energy was derived from fat, 20% from protein, and 60% from carbohydrates, with an energy density of approximately 3.1 kcal/g.

To induce metabolic dysfunction, animals in the diabetic groups received a high-fat diet in which approximately 60% of total energy was derived from fat. The primary fat source consisted of 90% processed animal fat and 10% soybean oil added to the basal animal diet (Axelsen et al., 2010). This dietary regimen was continued for 18 months to provide appropriate conditions for the development of obesity, insulin resistance, and impaired glucose metabolism.

In addition to the high-fat diet, the animals had *ad libitum* access to a 25% fructose solution. To prepare the solution, liquid fructose was dissolved in water, and a fresh solution was prepared daily and placed in animal drinking bottles (Lozano et al., 2016). The simultaneous consumption of a high-fat diet and fructose established a stable diet-induced model of type 2 diabetes that closely resembles metabolic abnormalities observed in humans.

2.4. Assessment of Metabolic Indices and Confirmation of Diabetes

To evaluate the successful establishment of the diabetes model, the animals' metabolic status was assessed at the end of the study period. Blood glucose concentration was measured using a glucometer. In addition, insulin resistance, as one of the principal indicators of impaired glucose metabolism, was calculated and used as a criterion for confirming diabetes in the animals.

Throughout the study period, body weight was measured weekly to monitor changes resulting from the dietary regimens and exercise interventions. Before final tissue sampling, the body weight and body length of each animal were also measured. In addition to monitoring animal growth, these measurements were used to evaluate metabolic alterations resulting from diet and exercise training.

2.5. Confirmation of Aging

To establish the aging condition, the animals were maintained until 21 months of age, which is considered an aged stage in Wistar rats. In addition to chronological age, senescence-associated β -galactosidase (SA- β -gal) activity was measured in cerebellar tissue as a widely used biochemical marker of cellular senescence. Increased activity of this enzyme was considered an indicator of senescent-cell accumulation and confirmation of cellular aging in the animals.

2.6. Treadmill Familiarization and Determination of Maximal Exercise Capacity

Before initiation of the exercise interventions, all animals underwent a treadmill familiarization period to reduce stress associated with the novel environment and minimize individual differences in learning to run. This period consisted of 10 sessions during which the animals ran for 5–

10 min/day at a speed of 8–10 m/min with a 0° incline on a rodent treadmill. In addition to facilitating adaptation to the exercise conditions, this stage enabled the identification of animals unable to perform the exercise protocol.

Exercise sessions were conducted using a rat-specific treadmill equipped with five separate lanes and an electric motor allowing precise adjustment of running speed. At the end of each lane, a metal grid connected to a 1-mA electrical stimulator was positioned and used only as a mild stimulus to encourage continued running when an animal stopped. Before the study began, the actual treadmill speed was verified by measuring the belt length and the time required for one complete revolution and comparing these measurements with the speed displayed by the treadmill to ensure accurate device operation.

Following the familiarization period, the maximal exercise capacity of each animal was determined individually. Each rat initially completed a 5-min warm-up at 8 m/min. Thereafter, treadmill speed was increased by 3 m/min every 3 min while the treadmill incline was maintained at 10°. The test continued until the animal returned to the end of the lane five consecutive times within 1 min and was no longer able to continue running. The highest recorded running speed was considered 100% of the maximal exercise capacity of that animal. The test was performed before initiation of training, at the end of each month, and again at the end of the 3-month exercise period so that exercise intensity could be adjusted according to changes in each animal's aerobic capacity (Qin et al., 2020).

2.7. Aerobic Exercise Protocols

After maximal exercise capacity had been determined, animals in the two intervention groups completed their respective exercise programs for 3 months, comprising a total of 40 training sessions. All exercise sessions were conducted toward the end of the animals' sleep cycle, between 16:00 and 18:00, to minimize the influence of circadian variations on physiological responses.

2.8. Moderate-Intensity Continuous Training (MICT)

Animals in the MICT group exercised at an intensity corresponding to 50%–70% of maximal exercise capacity. Each session lasted 34–42 min and was adjusted so that the overall exercise volume was equivalent to that of the HIIT

group. Each session consisted of a 5-min warm-up at 50% of maximal exercise capacity, a main continuous exercise phase at moderate intensity, and a 3-min cool-down at the same initial intensity. Session duration was adjusted according to the functional capacity of each animal to maintain a consistent training load throughout the study period (Haram et al., 2009).

2.9. High-Intensity Interval Training (HIIT)

Animals in the HIIT group also completed 40 exercise sessions using a high-intensity interval protocol. Exercise intensity during the high-intensity intervals was set at 80%–95% of maximal exercise capacity, whereas active recovery intervals were performed at 50%–70% of maximal exercise capacity. Each session consisted of three to four 4-min high-intensity intervals interspersed with two to three 3-min periods of lower-intensity activity. Total session duration ranged from 26 to 33 min. Similar to the MICT group, each session began with a 5-min warm-up and ended with a 3-min cool-down at 50% of maximal exercise capacity (Haram et al., 2009).

Both protocols were designed so that the overall exercise volume was approximately equivalent between groups, with exercise intensity representing the principal intervention variable. This design enabled direct comparison of the effects of exercise intensity on markers of autophagy and cellular senescence.

2.10. Tissue Sampling

Forty-eight hours after the final exercise session, the animals were anesthetized by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). The 48-hr interval between the final exercise session and tissue sampling was selected to minimize the acute effects of exercise and allow assessment of chronic exercise-induced adaptations.

After complete anesthesia had been achieved, cerebellar tissue was rapidly excised under sterile conditions. The samples were initially washed with cold normal saline and then divided into several portions. A portion of the tissue intended for measurement of autophagy-related proteins was immediately stored at −70°C.

2.11. Measurement of Molecular Markers

The expression levels of the autophagy-related proteins LC3-I, LC3-II, and Beclin-1 were determined using Western blotting. For this purpose, tissue samples were homogenized in lysis buffer and subsequently centrifuged. Total protein concentration was determined using the Bradford assay to ensure that an equal amount of protein was loaded into each well.

Following denaturation, protein samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto polyvinylidene fluoride (PVDF) membranes. Following blocking, the membranes were incubated with specific antibodies against LC3, Beclin-1, and GAPDH. Subsequently, after addition of the secondary antibody and chemiluminescent substrate, protein bands were detected using a digital imaging system. The intensity of each protein band was quantified using the dedicated imaging-system software and, after normalization to GAPDH, was reported as relative density. The LC3-II/LC3-I ratio was also calculated by dividing LC3-II band intensity by LC3-I band intensity and was used as the principal index of autophagic activity.

To confirm cellular senescence, SA- β -gal activity was measured in cerebellar tissue samples using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. Increased activity of this enzyme was considered an indicator of the accumulation of senescent cells.

According to the study design, only the four autophagy-related markers—LC3-I, LC3-II, Beclin-1, and the LC3-II/LC3-I ratio—together with SA- β -gal and the metabolic

indices of blood glucose and insulin resistance were evaluated. Other proteins examined in the original dissertation were not included in the analyses reported in the present article.

2.12. Statistical Analysis

Data were reported as mean $\pm$ standard deviation. Normality of data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. One-way analysis of variance (ANOVA) was used to compare differences among groups, and when a statistically significant difference was identified, Tukey's post hoc test was applied for pairwise comparisons. All statistical analyses were performed using SPSS version 22, and statistical significance was set at $p < .05$.

3. Findings and Results

To ensure the successful establishment of the animal model, indices associated with diabetes induction and aging were first examined in the control groups. The results of the Shapiro–Wilk test indicated that all study variables were normally distributed, and Levene's test confirmed homogeneity of variances among the control groups. Therefore, parametric tests were considered appropriate for between-group comparisons.

Assessment of the metabolic indices showed that blood glucose concentration was lowest in the young normal control group, whereas a substantial increase was observed in the young diabetic group. Blood glucose was also elevated in the aged healthy group compared with the young normal control group; however, the highest level was observed in the aged diabetic group.

Table 1

Descriptive Statistics for the Study Dependent Variables in the Control Groups

Dependent Variable	Group	Mean	SD
LC3-I	Young normal	1.36	0.03
	Young diabetic	1.64	0.01
	Aged healthy	1.87	0.04
	Aged diabetic	2.13	0.04
LC3-II	Young normal	0.66	0.31
	Young diabetic	0.23	0.006
	Aged healthy	0.29	0.006
	Aged diabetic	0.10	0.004
Beclin-1	Young normal	1.002	0.03
	Young diabetic	0.8005	0.03

SA- β -gal	Aged healthy	0.51	0.04
	Aged diabetic	0.219	0.04
	Young normal	21.74	1.07
	Young diabetic	43.07	1.96
LC3-II/LC3-I ratio	Aged healthy	62.14	2.56
	Aged diabetic	103.84	22.77
	Young normal	0.49	0.238
	Young diabetic	0.14	0.004
Body weight	Aged healthy	0.16	0.009
	Aged diabetic	0.05	0.003
	Young normal	220.50	0.50
	Young diabetic	331.00	3.00
Blood glucose	Aged healthy	361.00	64.00
	Aged diabetic	448.50	26.50
	Young normal	88.50	0.50
	Young diabetic	174.50	3.50
Insulin resistance	Aged healthy	150.50	6.50
	Aged diabetic	251.50	10.50
	Young normal	1.945	0.03
	Young diabetic	4.475	0.05
	Aged healthy	3.585	0.26
	Aged diabetic	8.795	0.40

The results of the one-way ANOVA showed statistically significant differences among the groups ($p < .001$), and Tukey's post hoc test further confirmed significant

differences between the aged diabetic group and the other groups.

Table 2

Results of One-Way ANOVA for Comparison of Dependent Variables Among the Control Groups

Dependent Variable	Sum of Squares	df	Mean Square	F	p	Effect Size
LC3-I	0.99	3	0.33	192.84	<.001	0.99
LC3-II	0.52	3	0.17	7.17	.012	0.73
Beclin-1	1.057	3	0.35	270.02	<.001	0.99
SA- β -gal	10967.46	3	3655.82	27.59	<.001	0.91
LC3-II/LC3-I ratio	0.34	3	0.11	7.922	.009	0.75
Body weight	79722.75	3	26574.25	22.11	.001	0.89
Blood glucose	40886.25	3	13628.75	330.39	.001	0.99
Insulin resistance index	76.96	3	25.65	458.70	.001	0.99

Table 3

Tukey Multiple Comparisons for the Dependent Variables Among the Control Groups

Variable	Comparison	Mean Difference	p
LC3-I	Young normal – Young diabetic	–0.28	<.001
	Young normal – Aged healthy	–0.51	<.001
	Young normal – Aged diabetic	–0.78	<.001
	Young diabetic – Aged healthy	–0.23	.001
	Young diabetic – Aged diabetic	–0.49	<.001
	Aged healthy – Aged diabetic	–0.26	<.001
LC3-II	Young normal – Young diabetic	0.43	.038
	Young normal – Aged healthy	0.37	.070
	Young normal – Aged diabetic	0.56	.010
	Young diabetic – Aged healthy	–0.06	.970
	Young diabetic – Aged diabetic	0.13	.740
	Aged healthy – Aged diabetic	0.19	.490

Beclin-1	Young normal – Young diabetic	0.202	.001
	Young normal – Aged healthy	0.49	<.001
	Young normal – Aged diabetic	0.79	<.001
	Young diabetic – Aged healthy	0.29	<.001
	Young diabetic – Aged diabetic	0.58	<.001
	Aged healthy – Aged diabetic	0.29	<.001
SA-β-gal	Young normal – Young diabetic	-21.33	.185
	Young normal – Aged healthy	-40.39	.011
	Young normal – Aged diabetic	-82.09	<.001
	Young diabetic – Aged healthy	-19.07	.250
	Young diabetic – Aged diabetic	-60.77	.001
	Aged healthy – Aged diabetic	-41.70	.009
LC3-II/LC3-I ratio	Young normal – Young diabetic	0.35	.029
	Young normal – Aged healthy	0.34	.035
	Young normal – Aged diabetic	0.44	.008
	Young diabetic – Aged healthy	-0.01	.990
	Young diabetic – Aged diabetic	0.09	.774
	Aged healthy – Aged diabetic	0.11	.702
Body weight	Young normal – Young diabetic	-110.50	.020
	Young normal – Aged healthy	-140.50	.010
	Young normal – Aged diabetic	-228.00	.001
	Young diabetic – Aged healthy	-30.00	.720
	Young diabetic – Aged diabetic	-117.50	.010
	Aged healthy – Aged diabetic	-87.50	.060
Blood glucose	Young normal – Young diabetic	-86.00	.001
	Young normal – Aged healthy	-62.00	.001
	Young normal – Aged diabetic	-163.00	.001
	Young diabetic – Aged healthy	24.00	.010
	Young diabetic – Aged diabetic	-77.00	.001
	Aged healthy – Aged diabetic	-101.00	.001
Insulin resistance index	Young normal – Young diabetic	-2.53	.001
	Young normal – Aged healthy	-1.64	.001
	Young normal – Aged diabetic	-6.85	.001
	Young diabetic – Aged healthy	0.89	.010
	Young diabetic – Aged diabetic	-4.32	.001
	Aged healthy – Aged diabetic	-5.21	.001

The insulin resistance index exhibited a pattern similar to that of blood glucose. The lowest value was observed in the young normal control group, whereas the highest value occurred in the aged diabetic group. ANOVA revealed a statistically significant difference among the groups ($p < .001$), and multiple comparisons indicated that both aging and diabetes independently increased insulin resistance, whereas their coexistence resulted in the greatest degree of insulin resistance.

To confirm the induction of cellular senescence, SA-β-gal activity was measured. As shown in Tables 1 and 2, the mean value of this marker progressively increased from the young normal control group to the aged diabetic group, with the aged diabetic group exhibiting the highest SA-β-gal activity. ANOVA confirmed a significant difference among the groups ($p < .001$), and Tukey's test showed that SA-β-gal

activity in the aged diabetic group was significantly higher than that in the young normal control group and the aged nondiabetic group (Tables 2 and 3).

In addition to metabolic and senescence-related indices, baseline autophagy-related markers were examined in the control groups. The results showed that LC3-I expression was highest in the aged diabetic group, whereas LC3-II expression, Beclin-1 expression, and the LC3-II/LC3-I ratio reached their lowest values in this group. The observed differences were statistically significant for all of these variables ($p < .05$), and Tukey's test further confirmed differences between the aged diabetic and young normal control groups.

Overall, the results of this section indicated that the experimental model successfully induced diabetes and aging in the animals. Significant increases in blood glucose, insulin

resistance, and SA- β -gal, together with alterations in autophagy-related markers, confirmed the suitability of the animal model for investigating the effects of exercise interventions on cerebellar autophagy.

To investigate the effects of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) on cerebellar autophagic status, changes in four principal markers of this pathway—LC3-I, LC3-II, Beclin-1, and the LC3-II/LC3-I ratio—were evaluated in the aged diabetic groups. Statistical analysis showed that both exercise modalities significantly altered these markers

compared with the aged diabetic control group, although the magnitude of the responses differed between the two exercise protocols for some markers.

Western blot findings and the quantitative data presented in Table 4 showed that the highest LC3-I protein expression was observed in the aged diabetic control group, whereas both exercise programs reduced this marker. Mean LC3-I expression was 2.13 ± 0.04 in the aged diabetic control group and decreased to 1.73 ± 0.08 after HIIT and to 1.57 ± 0.06 after MICT.

Table 4

Descriptive Statistics for the Study Dependent Variables in the Intervention Groups

Dependent Variable	Group	Mean	SD
LC3-I	Aged diabetic	2.13	0.04
	HIIT	1.73	0.08
	MICT	1.57	0.06
LC3-II	Aged diabetic	0.10	0.004
	HIIT	0.16	0.01
	MICT	0.20	0.01
Beclin-1	Aged diabetic	0.22	0.04
	HIIT	0.42	0.02
	MICT	0.58	0.01
SA- β -gal	Aged diabetic	103.84	22.77
	HIIT	79.39	5.06
	MICT	55.75	12.03
LC3-II/LC3-I ratio	Aged diabetic	0.05	0.003
	HIIT	0.09	0.004
	MICT	0.13	0.003
—	HIIT	0.72	0.03
	MICT	0.68	0.02
Blood glucose	Aged diabetic	251.50	6.06
	HIIT	199.00	12.17
	MICT	197.50	7.79
Insulin resistance	Aged diabetic	8.80	0.23
	HIIT	6.17	0.29
	MICT	5.71	0.38

One-way ANOVA indicated a statistically significant difference among the three groups, $F = 67.312$, $p < .001$, effect size = 0.96 (Table 5). Tukey's post hoc test (Table 6) further showed that both exercise programs significantly reduced LC3-I expression compared with the aged diabetic

control group ($p < .001$). In addition, the difference between the two exercise groups was statistically significant ($p = .040$), with MICT producing a greater reduction in LC3-I expression than HIIT.

Table 5

Results of One-Way ANOVA Comparing the Effects of Different Exercise Intensities on LC3-I

Dependent Variable	Sum of Squares	df	Mean Square	F	p	Effect Size
LC3-I	0.505	2	0.253	67.312	<.001	0.96

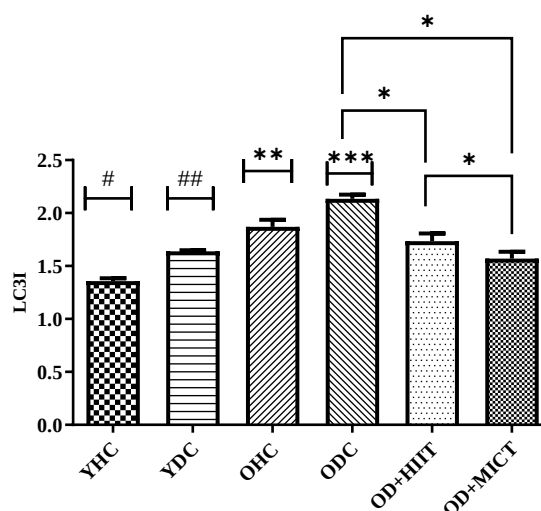
Table 6

Post Hoc Comparisons for Identifying Between-Group Differences in LC3-I

Dependent Variable	Comparison	Mean Difference	SE	p
LC3-I	Aged diabetic – HIIT	0.401	0.05	<.001
	Aged diabetic – MICT	0.564	0.05	<.001
	HIIT – MICT	0.163	0.05	.040

Figure 1

Changes in LC3-I protein expression levels across the study groups.



Evaluation of the Western blot results showed that the pattern of changes in LC3-II was opposite to that observed for LC3-I. The lowest LC3-II expression was observed in the aged diabetic control group, whereas both exercise modalities increased its expression. As shown in Table 4, mean LC3-II expression was 0.10 ± 0.004 in the aged diabetic control group and increased to 0.16 ± 0.01 following HIIT and 0.20 ± 0.01 following MICT.

One-way ANOVA revealed a statistically significant difference among the groups, $F = 60.630$, $p < .001$, effect size = 0.95 (Table 7). Tukey's post hoc test (Table 8) indicated that both exercise programs significantly increased LC3-II expression compared with the aged diabetic control group ($p < .001$). In addition, LC3-II expression was significantly higher in the MICT group than in the HIIT group ($p = .012$) (Figure 2).

Table 7

Results of One-Way ANOVA Comparing the Effects of Different Exercise Intensities on LC3-II

Dependent Variable	Sum of Squares	df	Mean Square	F	p	Effect Size
LC3-II	0.014	2	0.007	60.630	<.001	0.95

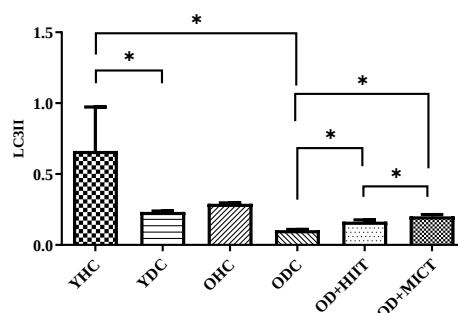
Table 8

Post Hoc Comparisons for Identifying Between-Group Differences in LC3-II

Dependent Variable	Comparison	Mean Difference	SE	p
LC3-II	Aged diabetic – HIIT	-0.059	0.0089221	.001
	Aged diabetic – MICT	-0.097	0.0089221	<.001
	HIIT – MICT	-0.038	0.0089221	.012

Figure 2

Changes in LC3-II protein expression levels across the study groups.



Comparison of Beclin-1 protein expression showed that the aged diabetic control group exhibited the lowest expression level, whereas both aerobic exercise protocols increased Beclin-1 expression. As shown in Table 4, mean Beclin-1 expression was 0.22 ± 0.04 in the aged diabetic control group, increasing to 0.42 ± 0.02 in the HIIT group and 0.58 ± 0.01 in the MICT group.

According to the ANOVA results (Table 9), the between-group difference was statistically significant, $F = 138.508$, p

$< .001$, effect size = 0.98. Post hoc comparisons (Table 10) showed that both exercise programs significantly increased Beclin-1 expression compared with the aged diabetic control group ($p < .001$). The difference between the two exercise modalities was also statistically significant ($p = .001$), with MICT producing a greater increase in Beclin-1 expression than HIIT (Figure 3).

Table 9

Results of One-Way ANOVA Comparing the Effects of Different Exercise Intensities on Beclin-1

Dependent Variable	Sum of Squares	df	Mean Square	F	p	Effect Size
Beclin-1	0.191	2	0.095	138.508	<.001	0.98

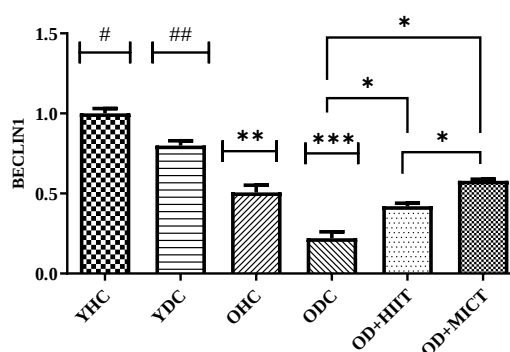
Table 10

Post Hoc Comparisons for Identifying Between-Group Differences in Beclin-1

Dependent Variable	Comparison	Mean Difference	SE	p
Beclin-1	Aged diabetic – HIIT	−0.201	0.021	<.001
	Aged diabetic – MICT	−0.356	0.021	<.001
	HIIT – MICT	−0.154	0.021	.001

Figure 3

Changes in Beclin-1 protein expression levels across the study groups.



To provide a more detailed evaluation of autophagic status, the LC3-II/LC3-I ratio was calculated for the three study groups. The results showed that this ratio was lowest in the aged diabetic control group and increased following both exercise programs. According to the data presented in Table 4, the mean LC3-II/LC3-I ratio was 0.05 ± 0.003 in the aged diabetic control group, whereas it increased to 0.09 ± 0.004 in the HIIT group and 0.13 ± 0.003 in the MICT group.

ANOVA results (Table 11) demonstrated a statistically significant difference among the three groups, $F = 404.147$, $p < .001$, effect size = 0.99. Tukey's post hoc test (Table 12)

further showed that both exercise programs significantly increased the LC3-II/LC3-I ratio compared with the aged diabetic control group ($p < .001$). The difference between the two exercise modalities was also statistically significant ($p < .001$), with MICT resulting in a higher ratio than HIIT (Figure 4). Overall, the findings of this section demonstrated that both exercise programs favorably modulated the principal markers of autophagy in the cerebellum of aged diabetic rats. However, for LC3-I, LC3-II, Beclin-1, and the LC3-II/LC3-I ratio, MICT produced more favorable and statistically significant changes than HIIT.

Table 11

Results of One-Way ANOVA Comparing the Effects of Different Exercise Intensities on the LC3-II/LC3-I Ratio

Dependent Variable	Sum of Squares	df	Mean Square	F	p	Effect Size
LC3-II/LC3-I ratio	0.010	2	0.005	404.147	<.001	0.99

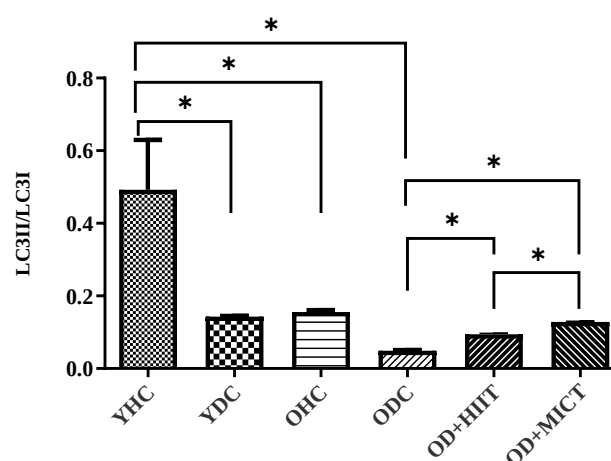
Table 12

Post Hoc Comparisons for Identifying Between-Group Differences in the LC3-II/LC3-I Ratio

Dependent Variable	Comparison	Mean Difference	SE	p
LC3-II/LC3-I	Aged diabetic – HIIT	−0.045	0.0028	<.001
	Aged diabetic – MICT	−0.079	0.0028	<.001
	HIIT – MICT	−0.034	0.0028	<.001

Figure 4

Changes in the LC3-II/LC3-I protein expression ratio across the study groups.



4. Discussion and Conclusion

The present study compared the effects of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) on cerebellar autophagy-related

markers and cellular senescence in aged rats with diet-induced diabetes. The principal findings showed that the combination of aging and diabetes was associated with a marked disturbance in the autophagic profile of cerebellar tissue, characterized by increased LC3-I expression, reduced

LC3-II expression, decreased Beclin-1 expression, and a lower LC3-II/LC3-I ratio. In parallel, senescence-associated β -galactosidase (SA- β -gal) activity, blood glucose, and insulin resistance were substantially elevated in aged diabetic animals. Both aerobic exercise interventions improved these abnormalities, but MICT produced more favorable changes than HIIT in LC3-I, LC3-II, Beclin-1, and the LC3-II/LC3-I ratio and also resulted in a greater reduction in SA- β -gal activity. These findings suggest that chronic aerobic exercise can attenuate diabetes- and aging-related impairment of cerebellar cellular homeostasis, while moderate continuous exercise may provide a more appropriate stimulus than high-intensity interval exercise for restoring autophagy in aged metabolically compromised neural tissue.

The observed deterioration in autophagy-related markers in the aged diabetic control group is consistent with evidence indicating that aging is accompanied by progressive impairment of intracellular quality-control mechanisms. Autophagy is a central mechanism through which long-lived cells remove damaged proteins, dysfunctional organelles, and potentially toxic intracellular material, and impairment of this pathway has been strongly linked to age-related cellular dysfunction (2, 5). In neural tissue, this issue is particularly important because neurons have limited regenerative capacity and depend heavily on effective proteostatic and lysosomal mechanisms to maintain long-term viability. Caponio et al. emphasized that both autophagy and mitophagy become compromised during brain aging, contributing to the accumulation of damaged mitochondria and abnormal proteins and thereby increasing susceptibility to neurodegenerative processes (4). Therefore, the lower LC3-II/LC3-I ratio and Beclin-1 expression observed in the aged diabetic rats in the present study can be interpreted as evidence of diminished autophagic competence in the cerebellum.

The cerebellum may be especially vulnerable to the combined effects of aging and metabolic dysfunction. Although traditionally examined mainly in relation to motor coordination, the cerebellum is now recognized as an important structure affected by age-related cellular and molecular disturbances. Aging-related changes in cerebellar neurons, glial cells, synapses, mitochondria, and intracellular signaling pathways may compromise the ability

of this region to respond effectively to metabolic stress (3). The general neuropathological effects of aging, including progressive structural and biochemical deterioration, have also been well documented in the brain (1). In this context, the present finding that aged diabetic rats exhibited the most adverse autophagic profile among the control groups supports the assumption that diabetes intensifies the vulnerability already created by normal aging.

The pronounced elevations in blood glucose and insulin resistance in the aged diabetic group further support this interpretation. Type 2 diabetes is associated with a range of adverse effects on the aging brain, including impaired cognition, altered brain structure, vascular dysfunction, and increased risk of neurodegenerative conditions (6). More recent evidence has demonstrated accelerated brain aging in individuals with diabetes and has linked poor glycemic control with more severe brain-aging phenotypes and greater mortality risk (7). Persistent hyperglycemia and insulin resistance can increase oxidative stress, mitochondrial dysfunction, inflammation, and abnormal nutrient-sensing signaling, all of which may interfere with autophagic regulation. Therefore, the interaction between diabetes and aging probably produces a cumulative metabolic burden that impairs the normal turnover of intracellular components and accelerates cellular degeneration in the cerebellum.

The dietary model used in the present study is also consistent with previous experimental evidence showing that long-term combined exposure to high-fat and high-fructose diets can reproduce major metabolic characteristics of type 2 diabetes. Axelsen et al. reported that prolonged high-fructose and high-fat feeding in rats leads to severe obesity, marked metabolic dysfunction, and extensive lipid accumulation (8). Similarly, Lozano et al. demonstrated that combined high-fructose and high-fat feeding produces substantial disturbances in glucose metabolism and increases the risk of diabetes and related systemic complications (9). The marked increases in glucose and insulin resistance observed in the current aged diabetic animals therefore confirm that the model generated the intended chronic metabolic stress and provide a plausible physiological basis for the observed cerebellar autophagy impairment.

Another important finding was the substantial elevation of SA- β -gal activity in the aged diabetic group. Cellular

senescence is a hallmark of biological aging and reflects the accumulation of cells with altered metabolic, inflammatory, and secretory phenotypes. Although SA- β -gal does not directly measure autophagy, increased activity of this marker in conjunction with impaired autophagy may indicate a cellular environment characterized by reduced damage clearance and enhanced senescence burden. Autophagic dysfunction can contribute to the persistence of damaged proteins and organelles, which in turn can promote oxidative stress and senescence-associated signaling. The close relationship between autophagy and healthy longevity has been strongly emphasized in experimental models, where maintenance of autophagic function is associated with improved cellular survival and delayed aging (5). Likewise, reviews of tissue-specific aging indicate that reduced autophagic efficiency is an important component of age-related dysfunction in the brain and other organs (2). Thus, the simultaneous reduction in the LC3-II/LC3-I ratio and increase in SA- β -gal in the present aged diabetic rats supports the possibility that impaired cellular clearance and increased senescence develop concurrently under combined aging and metabolic stress.

Both exercise protocols improved the measured autophagy-related markers compared with the aged diabetic control condition. This finding is consistent with the established role of exercise as a potent regulator of metabolic and intracellular homeostasis. Aerobic exercise improves glucose utilization, insulin sensitivity, mitochondrial function, and cellular energy balance, and these adaptations may indirectly support the restoration of autophagy. Human exercise studies have also demonstrated favorable changes in metabolic and inflammatory outcomes following structured aerobic activity, particularly in populations characterized by obesity and metabolic dysfunction (10, 11). Although these studies differ from the present animal model, they support the broader principle that repeated aerobic exercise can reduce the chronic metabolic burden associated with obesity and insulin resistance.

A more direct mechanistic explanation for the exercise-induced improvement in autophagy is provided by evidence that physical exercise activates lysosomal and autophagy-related signaling in the brain. Huang et al. reported that exercise enhances brain lysosomal function through activation of the AMPK-SIRT1-TFEB pathway (12). AMPK

acts as a sensor of cellular energy stress and can facilitate autophagy initiation, while SIRT1 participates in metabolic adaptation and stress resistance. TFEB is a major regulator of lysosomal biogenesis and autophagy-related gene expression. Exercise-induced activation of these pathways could therefore improve the capacity of neurons to remove damaged proteins and organelles. The reductions in LC3-I and increases in LC3-II, Beclin-1, and the LC3-II/LC3-I ratio observed in both exercise groups in the present study are broadly compatible with enhanced engagement of the autophagic machinery after chronic aerobic training.

The increase in Beclin-1 expression following exercise deserves particular attention. Beclin-1 is critically involved in the initiation and nucleation phases of autophagosome formation, and reduced expression may indicate impaired activation of the autophagic pathway. The significant increase in Beclin-1 after both MICT and HIIT therefore suggests improved capacity for autophagosome formation in trained animals. This interpretation is supported by evidence that autophagic signaling is strongly responsive to energetic stress, mitochondrial remodeling, and repeated exercise exposure (5, 12). Since diabetes and aging can suppress these regulatory mechanisms, repeated exercise may partially restore the molecular environment required for effective autophagic initiation.

The reduction in LC3-I together with the increase in LC3-II and in the LC3-II/LC3-I ratio after training is also consistent with greater conversion of LC3-I to the lipidated LC3-II form associated with autophagosomal membranes. Although interpretation of LC3 markers alone should be made cautiously because autophagy is a dynamic process, the overall pattern observed in the present study, particularly when considered alongside increased Beclin-1 and reduced SA- β -gal, supports an exercise-associated improvement in autophagy-related status. Previous literature has consistently identified autophagy as an important mediator of longevity and cellular adaptation to metabolic stress (2, 5). The present results extend this concept by demonstrating that chronic aerobic exercise can favorably influence these markers in cerebellar tissue under conditions of simultaneous aging and diabetes.

A central finding of the study was that MICT produced more favorable changes than HIIT across the principal autophagy-related outcomes. This result is noteworthy

because HIIT is often considered a powerful stimulus for metabolic and cardiovascular adaptation, but higher exercise intensity does not necessarily produce superior responses in all tissues or physiological conditions. Haram et al. showed that both aerobic interval training and continuous moderate exercise can improve metabolic abnormalities in rats with low aerobic capacity, while the pattern and magnitude of adaptation differ according to the exercise modality (13). The current findings similarly indicate that both modalities were beneficial but suggest that, in aged diabetic cerebellar tissue, sustained moderate-intensity exercise may provide a more favorable balance between metabolic stimulation and cellular stress.

One explanation for the superior response to MICT may be that moderate-intensity exercise provides sufficient energetic stimulation to activate AMPK-dependent and lysosomal pathways without imposing excessive mitochondrial stress. Aged diabetic neural tissue is already exposed to oxidative damage, insulin resistance, mitochondrial inefficiency, and impaired proteostasis. Under such conditions, repeated high-intensity exercise may produce a greater acute energetic and oxidative challenge than the tissue can optimally accommodate. Evidence from Zhang et al. supports this possibility, as HIIT was shown to induce dysregulation of mitochondrial respiratory complexes and mitophagy in the hippocampus of middle-aged mice under specific experimental conditions (15). This finding suggests that high-intensity exercise may, depending on age, tissue, and protocol characteristics, disturb rather than uniformly enhance mitochondrial quality-control mechanisms.

At the same time, the beneficial effects of HIIT observed in the present study are consistent with evidence that high-intensity exercise can improve neural mitochondrial clearance under diabetic conditions. Khosravi et al. reported that HIIT reduced tau and β -amyloid accumulation in rats with type 2 diabetes through improvements in lactate-dependent mitophagy (16). This aligns with the current observation that HIIT increased LC3-II, Beclin-1, and the LC3-II/LC3-I ratio compared with the aged diabetic control group. Therefore, the current results should not be interpreted as indicating that HIIT is ineffective. Rather, they suggest that while both protocols enhance autophagy-related

responses, MICT may produce a more favorable net adaptation in the cerebellum of aged diabetic rats.

Differences between the present results and findings favoring HIIT may be explained by several methodological and biological factors, including animal age, brain region, severity and duration of diabetes, exercise prescription, training volume, and the specific molecular outcomes examined. Studies involving younger animals or less severe metabolic dysfunction may allow greater tolerance of high-intensity workloads. By contrast, 18- to 21-month-old diabetic rats represent a physiologically vulnerable population in which recovery capacity is likely reduced. In addition, the cerebellum may respond differently from the hippocampus or cortex because of region-specific patterns of neuronal architecture, energy metabolism, and mitochondrial regulation (3). Consequently, exercise intensity should be considered within the context of tissue vulnerability rather than as an isolated determinant of efficacy.

The individualized prescription of exercise intensity used in the present study may also have contributed to the reliability of the comparison between MICT and HIIT. Exercise intensity was adjusted relative to maximal exercise capacity and reassessed during the intervention period. Qin et al. demonstrated the importance of determining maximal oxygen consumption and quantifying exercise intensity in untrained male Wistar rats to achieve physiologically meaningful workload standardization (14). Such standardization is especially important in aged animals because absolute running speeds may represent substantially different relative intensities among individuals. By periodically reassessing maximal capacity, the present protocol reduced the likelihood that training adaptations would progressively alter the intended exercise stimulus.

The greater reduction in SA- β -gal following MICT also supports the possibility that moderate continuous exercise may be better tolerated by aged diabetic neural tissue. Improvements in glycemic control and insulin resistance can reduce glucotoxicity, oxidative stress, and inflammatory signaling, which may in turn decrease factors promoting cellular senescence. Both exercise modalities lowered blood glucose and insulin resistance relative to the aged diabetic control group, but the overall pattern of autophagy and senescence markers favored MICT. This result is compatible

with the broader evidence that regular aerobic exercise improves metabolic regulation and reduces adverse inflammatory and obesity-related outcomes (10, 11). Improved systemic metabolism may therefore have contributed to the neural effects observed in the cerebellum.

Taken together, the present findings support an integrated interpretation in which aging and chronic diabetes impair cerebellar cellular quality control, while aerobic exercise partially restores this balance. The aged diabetic condition was associated with impaired autophagy-related signaling, greater cellular senescence, hyperglycemia, and insulin resistance. Exercise improved these outcomes, probably through overlapping mechanisms involving enhanced metabolic control, improved energy sensing, lysosomal activation, and mitochondrial quality control. These effects are consistent with the established importance of autophagy in healthy aging (5), the vulnerability of the aging brain and cerebellum to impaired proteostasis (1, 3, 4), and the capacity of exercise to activate pathways involved in lysosomal and mitochondrial turnover (12, 16). Importantly, the superior response to MICT indicates that the optimal exercise intensity for neural protection in advanced age and diabetes may not necessarily be the highest tolerable intensity.

A number of limitations should be considered when interpreting the present findings. First, autophagy was evaluated using LC3-I, LC3-II, Beclin-1, and the LC3-II/LC3-I ratio, but additional markers such as p62/SQSTM1, ATG proteins, lysosomal proteins, and direct measurements of autophagic flux were not included. Second, the study did not directly assess mitochondrial function, oxidative stress, neuroinflammation, neuronal survival, or cerebellar histopathology, which limits mechanistic interpretation of the molecular findings. Third, SA- β -gal was used as the principal marker of cellular senescence without confirmation by additional senescence-related proteins. Fourth, only male Wistar rats were investigated, and the findings may therefore not be directly generalizable to females or other animal models. Finally, the use of a prolonged high-fat/high-fructose dietary model produces a complex metabolic phenotype that may not reproduce every feature of human type 2 diabetes.

Future research should incorporate more comprehensive assessment of autophagic flux using lysosomal inhibition

approaches and additional molecular markers such as p62, ATG5, ATG7, LAMP1, TFEB, AMPK, mTOR, and SIRT1. Studies should also examine mitochondrial respiration, mitophagy, oxidative stress, inflammatory signaling, apoptosis, and cerebellar neuronal morphology to clarify the mechanisms underlying the differential effects of MICT and HIIT. Including both sexes, multiple age groups, and different stages of diabetes would help determine whether the optimal exercise intensity changes across the lifespan or with disease severity. Dose-response studies comparing several moderate- and high-intensity protocols while matching total exercise work would be particularly valuable, as would experiments combining molecular outcomes with motor coordination, balance, learning, and cognitive assessments.

From a practical perspective, the results suggest that regular aerobic exercise may be beneficial for preserving neural cellular homeostasis under conditions associated with aging and metabolic dysfunction. Moderate-intensity continuous exercise may be especially suitable when physiological reserve and recovery capacity are reduced, because it may provide sufficient metabolic and molecular stimulation without the greater acute stress imposed by high-intensity exercise. In applied settings, exercise programs for older individuals with impaired glucose regulation should prioritize progressive adaptation, individualized intensity prescription, adequate recovery, and long-term adherence rather than assuming that greater intensity will automatically produce superior outcomes. Although direct translation from animal models to humans requires caution, the present findings support the use of appropriately prescribed moderate aerobic exercise as a potentially effective component of strategies aimed at reducing the neural consequences of aging and diabetes.

Authors' Contributions

All authors contributed substantially to the study and to manuscript development, and all approved the final version.

Declaration

The authors declare that artificial intelligence tools were used only to assist with language editing, translation, and improvement of the manuscript's readability. All conceptualization, study design, data collection, data

analysis, interpretation of findings, and final approval of the manuscript were performed by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the content.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

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Declaration of Interest

The authors report no conflict of interest.

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Ethics Considerations

The study placed a high emphasis on ethical considerations. Informed consent obtained from all participants, ensuring they are fully aware of the nature of the study and their role in it. Confidentiality strictly maintained, with data anonymized to protect individual privacy. The study adhered to the ethical guidelines for research with human subjects as outlined in the Declaration of Helsinki.

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