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Effect of aerobic exercise as a treatment on type 2 diabetes mellitus with depression-like behavior zebrafish

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ABSTRACT

Background: Depression is the most known complication of type 2 diabetes mellitus (T2DM). Aerobic exercise improves glycemic control in T2DM, although the underlying mechanisms of comorbid depression-like behaviors in T2DM have not yet been fully elucidated.
Methods: 120 zebrafish were randomly assigned to four groups: Control, T2DM, T2DM + metformin, and T2DM + aerobic exercise. Then, all animals except the control group were fed with high glucose fairy shrimp (~40 g/kg/day) and exposed reserpine (40 μg/ml for 20 min) for 10 days. Here, behavioral tests were used for model verification. Following the verification, all groups were treated as before. Additionally, the T2DM + metformin group received metformin (~10.6 mg/kg/day) at the same time, while the T2DM + aerobic exercise group received aerobic exercise 30 min/day. Finally, blood glucose and behavioral tests, as well as protein and molecular levels were determined at Day 11 and 12.
Results: Aerobic exercise alleviated depressive-like behavior and enhanced the levels of antidepressant biomarkers (NE, 5-HIAA) in zebrafish after 10 consecutive days of exercise. Additionally, 10 consecutive days of aerobic exercise decreased the levels of inflammatory biomarkers (IFN-γ, IL-1, IL-4) and depressive biomarkers (cortisol). Meanwhile, it also aided in the reduction of CD11b, IL-6, IL-6R, and caspase-3 expression to combat the neuroinflammation induced by T2DM, mediated the BDNF-TrkB pathway, and increased Bcl-2/Bax levels.
Conclusion: Given the remarkable similarity in neurochemistry between humans and zebrafish, this study supports the effectiveness of aerobic exercise as clinical guidance in preventing and treating T2DM complicated with depression.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder associated with genetics and diet, with its main clinical manifestation being chronic hyperglycemia [1]. Given the long-term increase in blood glucose, T2DM damages the macrovascular, micrangium [2] and endangers the heart [3] and brain [4]. According to the World Health Organization, diabetes complications were expected to reach more than 100 by 2020 [5], thereby making it the disease with the highest number of known complications. T2DM exerts an adverse effect on the central nervous system (CNS), where it induces neuroinflammation and consequently results in mental disorders such as depression, Alzheimer's disease, and epilepsy [6]. Depression often lasts many years, which also globally become a major human blight [7]. Studies have shown that T2DM doubles the risk of depression implying a complex pathogenetic relationship between T2DM and mental disorders [8,9]. The association suggests an etiology characterized by multiple complex interactions among multiple variables, including neuronal damage [10] and neurohormonal dysregulation, inflammation [11] and hippocampal structural alterations [12].

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Considering that zebrafish (*Danio rerio*) models are indispensable for studying the relationship between T2DM and mental disorders, and they have been successfully developed in neurodegenerative [13] and neuropharmacology [14,15] due to their numerous advantages, which include lower economic costs and small size organisms, lineal homologs of genes, and detailed anatomical descriptions [16]. Reserpine was used to induce depression-like behavioral zebrafish due to its side effects [17,18], help to induce diabetes mellitus complicated by depression model in zebrafish. Given that zebrafish shares 70% of its genome with humans [19], retains more than 80% of disease proteins [20], it has been widely used in the development of disease models, gene function evaluation [21–23], drug screening [24,25], toxicity detection [26,27], and risk assessment for environmental health [28,29]. Simultaneously, zebrafish investigations have benefited from the study and development of traditional Chinese medicine [30] and health food [31,32]. Notably, some critical brain regions in zebrafish perform the same function as they do in humans. For example, the zebrafish hypothalamic-pituitary-interrenal (HPI) axis, like human hypothalamic-pituitary-adrenal (HPA) axis, uses cortisol as the main stress hormone [33], the cerebral cortex of zebrafish telencephalic executes memory, the hippocampus and amygdala cause the starling response [34] and the dopaminergic system is equivalent to the human substantia nigra in the ventral midbrain. Using zebrafish models to understand the critical role of social interactions in mental health and wellbeing has become a reality [35]. Recently, Karl et al. successfully used non-invasive in vivo imaging techniques to identify specific neural signals during zebrafish sleep, the first neuronal sleep marker in zebrafish, and found that zebrafish undergo sleep in stages similar to mammals [36], implying a high similarity between zebrafish and humans. Collectively, the findings gathered in all of the above-mentioned studies suggest that using the zebrafish model provides useful guidance for humans. Over the years, numerous approaches for inducing T2DM models in zebrafish have been established [37], including a high-fat diet that when combined with overnutrition effectively mimics the characteristics of T2DM in humans, whereas medication exposure results in extremely high mortality [38].

Although metformin is a less expensive option for treating T2DM compared to other drugs, the side effects and burden on the kidney are still inevitable. Consequently, an increasing number of studies have advocated for exercise therapy as a means of alleviating diabetes [39–45], which may be related to the fact that it induces neurogenesis [46]. A recent study showed that only unlimited voluntary exercise stimulates the serotonergic system and suppresses depression-like behavior [47], thus, zebrafish may be a suitable model for voluntary exercise research due to their congenital countercurrent swimming characteristics. It should be noted that zebrafish is maturing as an animal model for movement disorder drug discovery [48]. Although the association between different types of exercise training interventions and glucose control is unknown, regular exercise improves glucose control in diabetes [49]. Moderate-intensity continuous training has been shown to improve glycemic control in middle-aged and older patients with T2DM [50]. Additionally, adults with T2DM may benefit from at least 150 min of aerobic exercise every week (at least 30 min per day, five days per week) [51]. Depression has been found to be associated with lower levels of aerobic exercise in patients with T2DM [52,53]. Biomedical engineering promotes the development of zebrafish aerobic exercise equipment [54,55]. However, the equipment was unable to deal with the large volume of the sample. Additionally, the underlying mechanisms of exercise in depression-like behavior with T2DM remain poorly understood.

As illustrated in the schematic diagram (Fig. 1), behavioral tests were first to demonstrated whether model was successfully established on zebrafish. Following the model verification, to determine whether

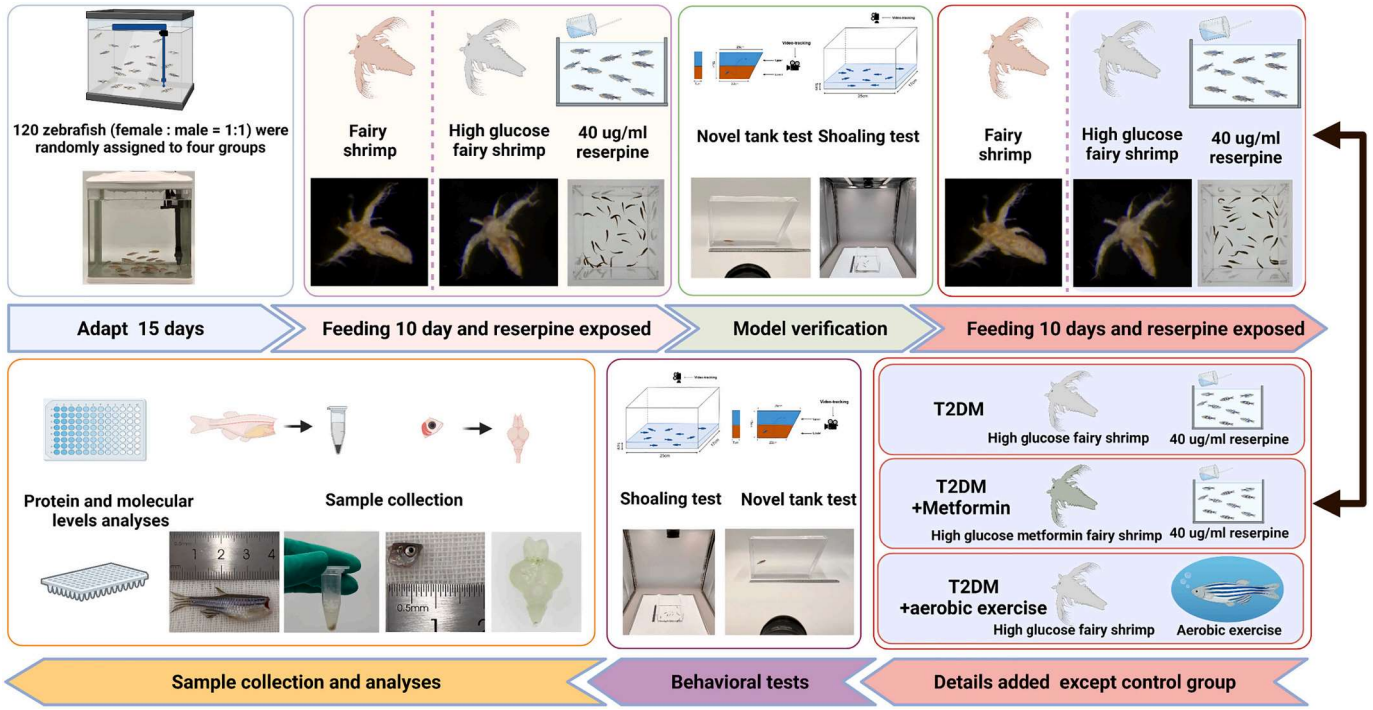

Fig. 1. Brief summary route of the research design. Here, zebrafish (female: male = 1:1), 30 per group, were initially placed into four tanks and divided into four groups (control, T2DM, T2DM + metformin, T2DM + aerobic exercise) and adapt 15 days in the tank. In order to established zebrafish T2DM with depression model, control group were fed with fairy shrimp for 10 days, while other groups were fed with high glucose fairy shrimp and exposed 40 μg/ml reserpine (20 min/day). Then, behavioral tests including NTT and shoaling test were here to verify model establishment. Following the verification, control, T2DM and T2DM + aerobic exercise group continue feeding as before for 10 days, T2DM + metformin fed with high glucose metformin fairy shrimp, while zebrafish in T2DM + aerobic exercise group were do aerobic exercise (30 min/day). Finally, follow the behavioral tests, zebrafish were decapitated and brains extracted for sample analyses. T2DM = zebrafish type 2 diabetes mellitus model; T2DM + metformin = zebrafish fed with high glucose metformin fairy shrimp; T2DM + aerobic exercise = zebrafish fed with high glucose fairy shrimp and also subjected to aerobic exercise (30 min/day). NTT = novel tank test.

aerobic exercise influences depression-like behavior in T2DM zebrafish, blood glucose and behavioral approaches, as well as protein and molecular levels, were analyzed in adult zebrafish. Moreover, our team demonstrated a novel approach for inducing T2DM complicated with depression in zebrafish. Additionally, we constructed aerobic exercise equipment for zebrafish.

2. Materials and methods

2.1. Experimental animals

Wild-type zebrafish (1:1 male-to-female ratio, purchased from Zebrafish resource center, Wuhan, China) were housed in a five-storey single-row Aquatic System (Haisheng Marine Biological Equipment Co., Shanghai, China) with a 14 h light:10 h dark cycle at a temperature of 28.5 ± 1 °C. The animals (adult zebrafish, 5 months old) were initially housed in 11-L Benchtop Aquatic Systems (Sensen Marine Biological Equipment Co., Jiangsu, China) and divided into three groups; control group, T2DM group, and T2DM + metformin group. The fourth group (T2DM + aerobic exercise) was housed in the self-assembled aquatic system's restroom (Fig. 2). Additionally, the environmental conditions including light cycle and temperature, were identical to those before the transition.

2.2. Fodder treatment

Unhatched fairy shrimp were purchased from a commercial supplier (Artemia Cysts, Fengnian Aquaculture Co., Tianjin, China). 40 g artemia cysts were symmetrically divided into two 2 L incubators (Jiankeshan Technology Co., Ltd. Jiangsu, China). Then, 1.5 L ddH_2O and 8 g salt (Fengnian Aquaculture Co., Tianjin, China) for incubation were decanted into each incubator. 16 h later, to establish a high glucose fairy shrimp fodder (Fig. S1B), one of the incubators was decanted with 500 ml 80% glucose (50–99-7, Aladdin, Shanghai, China) to a final concentration of 20%, while the other was decanted with 500 ml ddH_2O for fodder fed control group (Fig. S1C). The hatched fairy shrimps were collected 24 h after incubation and rinsed twice with ddH_2O. Then fairy shrimps were immediately placed in liquid nitrogen. Following that, a lyophilizer (SCIENTZ-12N, Xinzhi Biotechnology Co., Ltd., Zhejiang, China) was required for 36–48 h. Zebrafish except control group were fed with high glucose fairy shrimp at ~40 g/kg/day on an average weight of 0.5 g per adult zebrafish.

2.3. Training protocol

The equipment for zebrafish aerobic exercise was developed as described by Gilbert M et al. [55] Notably, dorsal body exposure and tank change are the schedules included in the zebrafish chronic unpredictable mild stress (CUMS) test to induce depression in zebrafish. The tank was divided into two houses (one for living and the other one for exercise training) separated by a door. Following that, we investigated the fate of zebrafish exposed to aerobic exercise (12 cm/s at maximum, 30 min per day for 10 days). We selected designated animals (n = 30 zebrafish), similar body mass index (BMI), to do aerobic exercise in three lane of the tank (40 length × 40 width × 30 height cm) (Fig. 2). Zebrafish were initially housed in the rest room (11 L water) (Fig. 2A). A self-assembly aquatic system was established in the restroom to maintain the same environment as the other groups, including a filtration system (Fig. 2B) and a temperature-controlled device (Fig. 2C). Before the experiment, a stick was used to drive the zebrafish along the swimming lane (Fig. 2D).

2.4. Drug treatment

Zebrafish in T2DM + metformin group were treated with metformin-HCl (1115-70-4, Sigma-Aldrich, Darmstadt, Germany) (~10.6 mg/kg/day) [56] which symmetrically combines with high glucose fairy shrimp fodder. Specific, fodder and metformin aqueous solution mix in a ratio of 20: 5.3 (g/mg), then lyophilized again (Fig. S1D). Because metformin is a drug commonly prescribed to treat patients with T2DM, metformin was used as the positive control in our study. Moreover, all animals except the control group were exposed 40 μg/ml reserpine (50–55-5, Sigma-Aldrich, Darmstadt, Germany) 20 min/day.

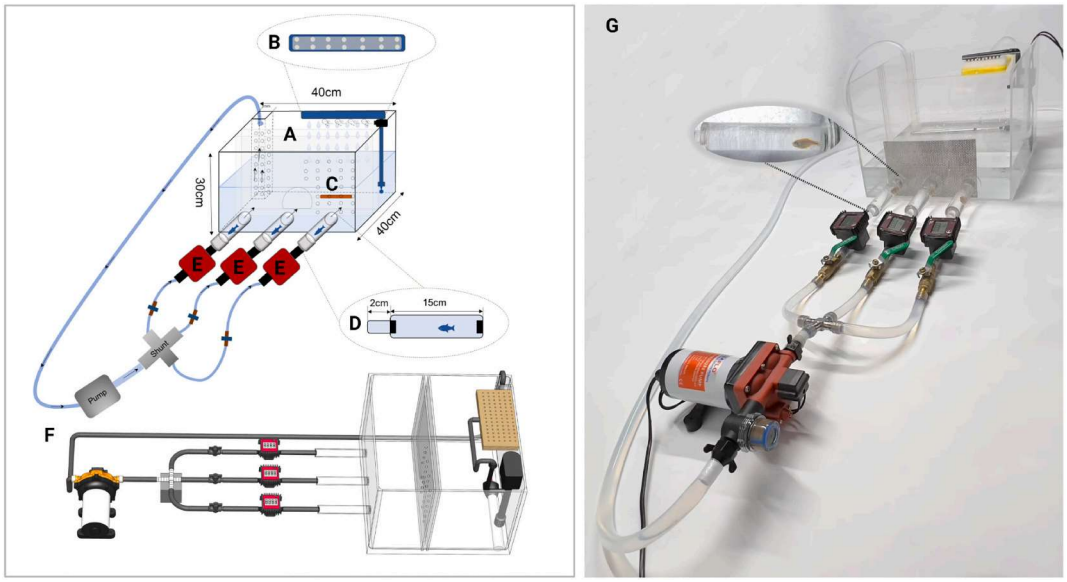

Fig. 2. Zebrafish aerobic exercise equipment. Inspiration from zebrafish congenital countercurrent swimming characteristics. Direction of arrows represents the water flow. Water was changed every 3 days through the pump, same as in other groups' tanks. (A) Zebrafish rest room. Zebrafish in T2DM+ aerobic exercise group were fed with fodder here. (B) Self-assembly aquatic system, needs to be replaced with a new filter cotton every day. (C) Temperature-controlled device, maintained the temperature at 28.5 ± 1 °C in the rest room. (D) Zebrafish swimming lane. Turn on the pump when zebrafish are in the middle of the lane. (E) Flowmeter, control valve to display 12 cm/s. F. Equipment 3 D model diagram use solidworks (Dassault Systemes S.A, Massachusetts, USA). G. Physical picture display.

2.5. Experimental design and sample collection

All groups of zebrafish should adapt in their tanks for 15 days before the experiment. After the adaption period, control group were fed twice a day (at 8: 30 AM and 5: 30 PM) with the fairy shrimp fodder without glucose, while the other groups received high glucose fairy shrimp fodder and exposed 40 μg/ml reserpine (20 min/day). The water in each tank was replaced 2/3 every 3 days through a pump, which protected the zebrafish from being startled. After feeding for 10 days, behavioral tests were performed to validate the model establishment. Following the verification, zebrafish in control, T2DM and T2DM + aerobic exercise group were continue fed and exposed in reserpine as before for 10 days, and T2DM + metformin group fed with high glucose metformin fairy shrimp, while zebrafish in T2DM + aerobic exercise group were also do aerobic exercise (30 min/day). Notably, the length and weight of each zebrafish's body were first determined to calculate the BMI, which is calculated as the body weight (g) divided by the square of the body length (cm). Following the behavior tests, zebrafish were first anesthetized in ice water (4 °C) [57], the surface was dried, blood glucose and BMI were determined, and the fish were decapitated and their brains extracted. The brain and body samples were immediately placed in liquid nitrogen then transferred to a −80 °C freezer for further analyses.

2.6. Behavioral testing

Behavioral testing was performed using the novel tank test (NTT) and the shoaling test. Briefly, the NTT was a trapezoidal tank (1.5 L, 15 height × 28 top/22 bottom × 7 width cm) that was essentially divided into top and lower halves [58]. The shoaling test was a foursquare tank (4 L, 10 height × 20 lengths × 20 width cm), with a water depth of 2 cm. Behavioral testing to determine the frequency of top transitions, time in the top (s), the latency to enter the top (s) in NTT as well as the distance between zebrafish (cm), and velocity (mm/s) in the shoaling test. Six distinct cameras were used to capture behavior tests, which were analyzed using Fish Track (Zebrafish Analysis Software, XinRuan Information Technology Co., Ltd. Shanghai, China).

2.7. Whole-body biochemical analyses

Blood glucose was measured using a micro-glucose meter (ACCU-CHEK, Switzerland, Basel). First, a microstrip (Instant 5, Switzerland, Basel) was prepared and inserted into the blood glucose meter. To obtain the blood, a scalpel was used to cut between the anal and caudal fins as described by Gabriela et al. [57]. The blood began to flow, and the microstrip was quickly dipped in the blood. Blood glucose levels were displayed after 5–9 s. Following BMI and blood tests, decapitation, and brain extraction, the headless body sample and head was respectively homogenized with 0.9% normal saline (1:9 weight/weight) and centrifuged for 20 min at 4 °C, 2500 rpm, to collect the supernatant for biochemical analyses. The levels of triglyceride (TG), total cholesterol (T-CHO), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein (LDL-C), and total protein (TP) in these whole-body samples were determined using biochemical kits (Jiancheng Company, Nanjing, China) according to the manufacturer's instructions. Additionally, whole-body cortisol, Interleukin-1 (IL-1) and Interleukin-4 (IL-4), IFN-γ, norepinephrine (NE), and 5-hydroxyindole acetic acid (5-HIAA) levels were determined using ELISA kits (ZIKE Biotech, Shenzhen, China) according to the manufacture's protocol. All data were normalized using the using BCA assay for total protein concentrations.

2.8. Quantitative real-time polymerase chain reaction (qRT-PCR)

To extract total RNA, Trisol (Takara, Japan) was used to lysate the homogenized whole-body muscle samples or whole-brain samples, according to the manufacturer's instructions. Notably, reverse transcription was carried out immediately after total mRNA extraction to avoid mRNA degradation. Following that, 1 μl total mRNA was reverse transcribed to cDNA using HiScript® III-RT SuperMix for qPCR (+gDNA wiper) (Vazyme Biotech Co., Nanjing, China) in accordance with the manufacturer's protocol, followed by preparation of fivefold dilutions of the cDNA samples. Finally, a 2 μl final cDNA sample was prepared for qRT-PCR using the LightCycler® 96 system (Roche, USA) and specific primers (Table 1) equipped with SYBR green fluorescent dyes (Vazyme Biotech Co., Nanjing, China). Relative mRNA expressions were normalized to the mRNA expression of the housekeeping gene β-actin and calculated using the ΔΔCT correction method [59].

The headless whole-body samples were eviscerated and residual muscle tissue samples were used to evaluate the expression of T2DM-related genes, including glucagon and insulin. This study examined the effect of aerobic exercise on depression-like behaviors in T2DM zebrafish because CNS-related gene expression is mainly found in the brain. Therefore, whole-brain samples were used to assess depression-related genes in this study. BDNF is associated with depression and antidepressants, and numerous preclinical [60,61] and clinical studies [62,63] have found that the BDNF signaling pathway, which is mediated by the TrkB and P75 receptors is impaired in the pathophysiology of mood disorders. Additionally, the expression of a microglia biomarker (CD11b), pro-inflammatory biomarkers (IL-1β, IL-6, and its receptor, IL-6R), and apoptotic genes caspase-3, Bcl-2, and Bax were determined in this study.

2.9. Statistics analyses and reproducibility

All statistical analyses were performed using GraphPad Prism (GraphPad Software Inc. San Diego, California, USA). All data are expressed as group means ± SEM or as box plots indicating the median. Parametric tests, such as the two-tailed unpaired *t*-test and ordinary one-way analysis of variance (ANOVA) followed by Tukey's post hoc test or Dunnett's multiple comparison test, were used to analyze the mean differences between groups. Significance levels indicated in all figures were as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Recognizing the high degree of variability among groups in animal experiments, individual values were presented in the diagram as dots of varying colors. Additionally, the n values indicate the total number of

Table 1
Primers used in the present study (Sangon Biotech, Shanghai, China).

Genes	Sequences (5′-3′)
Glucagon F	AAGCGAGGAGACGATCCAAA
Glucagon R	TCCAACACACACCAGCAAATG
Insulin F	GAGCCCCTTCTGGGTTTCC
Insulin R	AAGTCAGCCACCTCAGTTTCCT
BDNF F	GAGCTCAGCGTTTGTGACAG
BDNF R	GTCTGGCCCGACATGTCTAT
TrkB F	CACCAAGCTGGCATACATTG
TrkB R	AATGACGAAACGTCCTCCAG
P75 F	AGTGACGACAAACGCACAAG
P75 R	CAGAATCAGCAAAACCAGCA
CD11b F	TCCTCGGATTCCAGAAACAC
CD11b R	AGCAGCACAAGTCCTCCAAT
IL-1β F	GCTGGAGATCCAAACGGATA
IL-1β R	ATACGCGGTGCTGATAAACC
IL-6R	TCTTGGGGTCTTTCCCTCTT
IL-6F	TCAACTTCTCCAGCGTGATG
IL-6R R	ACTGACAGCACGCAAAACTC
IL-6R F	GCCAACTGCAACATACCAAA
Caspase-3 F	CCAGGGTCAACCATAAAGTAGC
Caspase-3 R	TCTTTGGTGAGCATTGAGACGA
Bcl-2 F	TGGATGACTGACTACCTGAAC
Bcl-2 R	GTATGAAAACGGGTGGAAC
Bax F	GTGTATGAGCGTGTTCGTC
Bax R	CGGCTGAAGATTAGAGTTGT
Actin F	CATCAGGGTGTCATGGTTGGT
Actin R	TCTCTTGCTCTGAGCCTCATCA

validated zebrafish in each group. Because of the importance of reproducibility in biomedical studies [64], all experiments were repeated 3 times independently by 3 different researchers.

3. Results

3.1. Zebrafish fed with high glucose fairy shrimp fodder exhibit the T2DM phenotype

T2DM impairs glucose metabolism dysfunction and promotes inflammation [65]. Persistent hyperglycemia is a significant feature of T2DM and bears remarkable similarities to clinical obesity caused by a high glucose intake. The BMI has been established as a screening paradigm for diabetes risk assessment [66]. We initially examined if high glucose fairy shrimp and reserpine-expose could induce the T2DM model in zebrafish. The zebrafish were housed in 11 L tanks and fed with high glucose fairy shrimp (twice a day) and exposed in 40 μg/ml reserpine (20 min/day) for 10 days to induce T2DM. We observed that zebrafish show higher blood glucose (Fig. 3A) and BMI (Fig. 3B) levels as expected. Additionally, a high glucose fairy shrimp diet significantly increases biochemical factors of T2DM including TG (Fig. 3D) and T-CHO (Fig. 3E), LDL-C (Fig. 3F), and TP (Fig. 3G), but had significantly decreased insulin (Fig. 3C) and HDL-C (Fig. 3F) levels. Moreover, this diet-induced higher levels of inflammation in T2DM zebrafish. The whole-body levels of IL-1 (Fig. 3I), IL-4 (Fig. 3J) (anti-inflammatory cytokine interleukin) and IFN-γ (Fig. 3K) (pro-inflammatory interferon-gamma) were elevated in the T2DM group. Additionally, biomarkers in T2DM zebrafish confirmed that glucagon (a diabetes mellitus biomarker) (Fig. 7A) expression was significantly higher than in other groups, but not insulin expression (Fig. 7B).

3.2. Reserpine and high glucose fairy shrimp diet induced depression-like behavior in zebrafish

To validate diabetes zebrafish models with depression-like behavior, we also analyzed the levels of depression-related biochemical factors, such as cortisol, NE, 5-HIAA, as well as molecular and behavioral tests as described by Jia et al. [11]. This study revealed that zebrafish exposed in 40 μg/ml reserpine significantly elevated cortisol (Fig. 4A) in zebrafish with T2DM, but decreased NE (Fig. 4B) and 5-HIAA levels (Fig. 4C).

In psychiatry, the zebrafish model has facilitated the development of behavioral analysis techniques [67]. The NTT [58,68], shoaling test [69], light/dark tank test [70], acoustic starling response [71], video-tracking experiment [72], and novel object approach test were all used to study depression-like behavior in zebrafish [73]. More importantly, zebrafish are active during the day [74]. Additionally, HPA axis stress is directly associated with depression, because the HPA axis is regulated by circadian rhythms. Above all, behavioral tests should be performed in the afternoon (5–8 h following light exposure) [75]. Gravity is specified as position in the NTT test for zebrafish. The fish was transferred to the new tank and kept for 15 min to adapt to the new environment as described by Egan, R.J et al. [76]. The shoaling test successfully distinguished between depression and depression behaviors [69]. The distance between zebrafish and swimming velocity was measured, and an increase in the distance between zebrafish significantly indicated depression. In NTT (Fig. 5) (Video S1), this study observed that T2DM-depressed zebrafish had a decreased transition to the top (Fig. 5D) and a shorter time on the top (Fig. 5E). Additionally, the latency to top (Fig. 5F) was longer in the control group than in the experimental group, which is consistent with clinical data [77]. In the shoaling test (Fig. 6) (Video S2), T2DM-depression zebrafish exhibited a higher velocity (Fig. 6D), while a longer distance between T2DM-depression zebrafish (Fig. 6E) was associated with depression.

Depression and neuroinflammation biomarkers such as CD11b (a microglial biomarker) (Fig. 7C), pro-inflammatory cytokines IL-6 (Fig. 7D), IL-6R (Fig. 7E), IL-1β (Fig. 7F) are significantly higher in the T2DM groups compared to those in the control group. Similarly, the levels of the brain-derived neurotrophic factor (BDNF) (Fig. 7G), its receptors p75 (Fig. 7H), TrkB (Fig. 7I), as well as apoptotic caspase-3 (Fig. 7J) and Bax (Fig. 7M) (but not Bcl-2) (Fig. 7K) genes were higher in the T2DM groups relative to the control group. Also, Bcl-2/Bax (Fig. 7L) was significantly lower in the zebrafish diabetes mellitus depression model than in the control group.

3.3. Aerobic exercise relieves elevated blood glucose and BMI

Exercise increases insulin sensitivity and synergistically increases glucose utilization in skeletal muscle [78]. Studies have shown that insulin sensitivity and endothelial function are worse in the evening than that in the morning. Here, the body seems to have glucose intolerance [79]. Therefore, it is plausible to expect that exercise in the afternoon could be better against decreased insulin sensitivity and endothelial function. Preliminary results from a recent study suggested that exercising in the afternoon was more effective in reducing glucose levels than in the morning [80]. As showing in Fig. 3, we were surprised to discover that exercise (blue) reduced blood glucose levels (Fig. 3A) and stabilized BMI (Fig. 3B), implying the feasibility of equipment and laying the foundation for later confirmation.

3.4. Aerobic exercise improves carbohydrate metabolism in T2DM zebrafish

The whole-body biochemical analyses were performed. Fig. 3 shows the anti-inflammatory and antidepressant effects of aerobic exercise in T2DM zebrafish. Aerobic exercise significantly decreased TG (Fig. 3D) and T-CHO levels (Fig. 3E) when compared to the T2DM group, even if it's not as effective as metformin. Surprisingly, aerobic exercise was more effective with regard to LDL-C (Fig. 3F) and HDL-C levels (Fig. 3G) than metformin, as well as insulin (Fig. 3C). Furthermore, swimming exercise increased TP (Fig. 3H) in vivo, which could be related to additional protein needed for muscle to fight against campaigns [81]. Fig. 4I–K shows that aerobic exercise had a higher anti-inflammatory effect than metformin in T2DM zebrafish. The whole-body levels of IL-1 (Fig. 3I), IL-4 (Fig. 3J), and IFN-γ (Fig. 3K) were under control. We demonstrated that aerobic exercise is beneficial to T2DM, and improves carbohydrate metabolism.

3.5. Aerobic exercise effectively reduces depression-like behaviors in T2DM zebrafish

This study employed a variety of approaches to explore the mechanisms underlying aerobic exercise's ability to alleviate depression-like behaviors in T2DM zebrafish, we employ a variety of approaches. Firstly, whole-brain biochemical analyses (Fig. 4) revealed that aerobic exercise decreased cortisol (Fig. 4A) and increased NE (Fig. 4B) and 5-HIAA levels (Fig. 4C) in comparison to the T2DM group. Although aerobic exercise did not exert the same physiological effect as metformin, it significantly reduced depression-like behavior in the NTT and shoaling test. Secondly, our research observed that aerobic exercise showed more transition to top (Fig. 5D) and a longer time in top (Fig. 5E) in NTT as effective as metformin. Additionally, exercise had little effect on the latency to top (Fig. 5F), which was consistent with previous clinical data [82]. In the shoaling test, exercise helped to reduce depression (Fig. 6D) and elevated the tendency to socialize with other companions (Fig. 6E). Finally, muscle tissue was chosen for analyses due to its glucose metabolism, whereas whole-brain samples were used for gene expression analyses due to the antidepressant mechanism of aerobic exercise in zebrafish. Exercise appeared to have a limited effect on gene expression (Fig. 7). Specifically, compared to control group, aerobic exercise was not affected insulin (Fig. 7B) and IL-6R (Fig. 7E), IL-1β (Fig. 7F), BDNF (Fig. 7G), p75 (Fig. 7H), and caspase-3 (Fig. 7J) levels, as effective as metformin. However, metformin promoted the expression

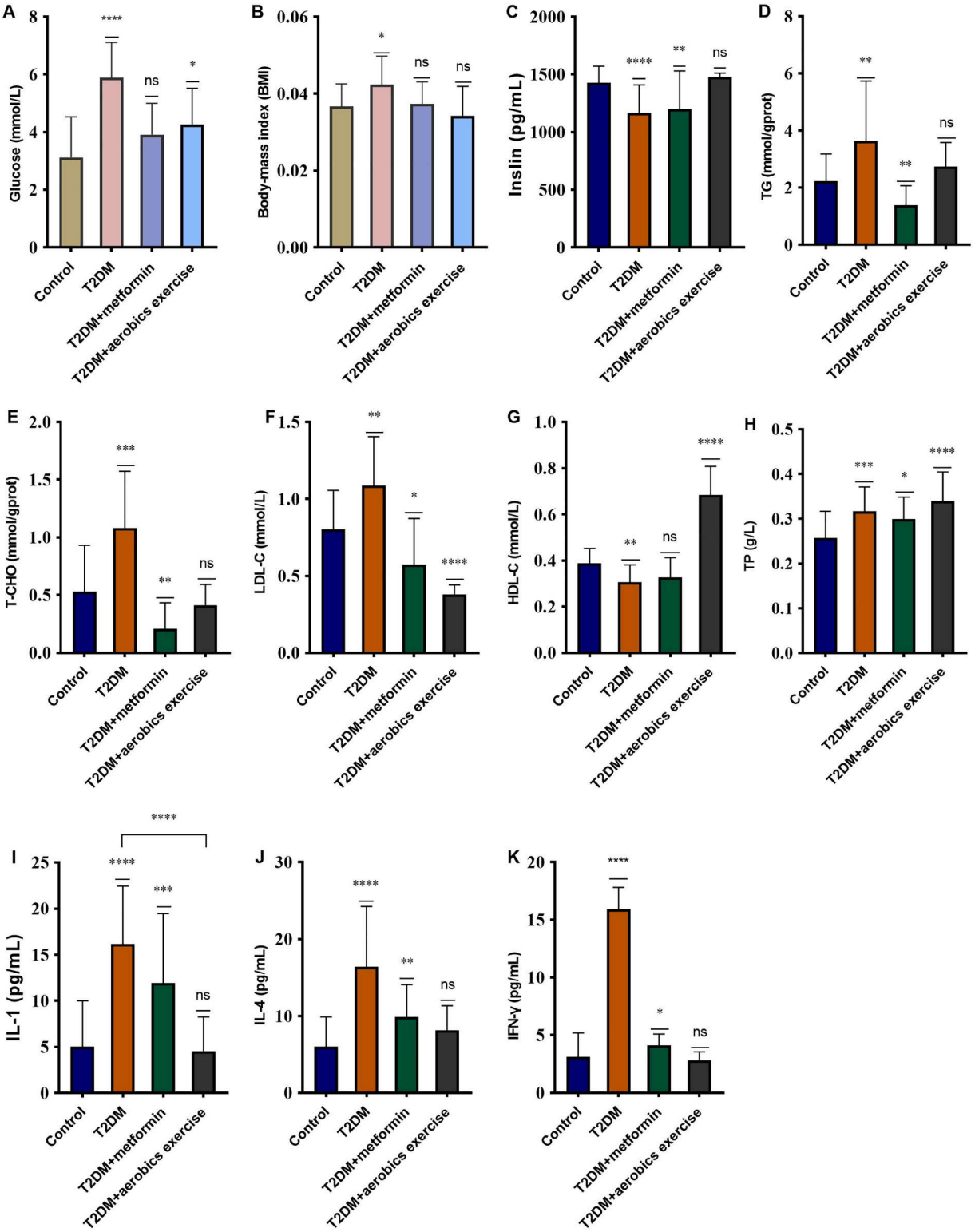

Fig. 3. Whole-body tissue examination and the levels of inflammation factors in different groups of zebrafish. (A) The level of glucose. Aerobic exercise exhibited the same effect as metformin ($p = 0.3976$), that is, it significantly attenuated the elevated glucose level caused by diabetes. (B) The level of BMI. (C) Insulin. (D) TG. (E) TCHO. (F) LDL-C. (G) HDL-C. (H) TP. (I) IL-1, aerobic exercise effectively decreased the level of IL-1 ($p < 0.0001$, vs T2DM group) and reduced the risk of inflammation associated with DM. (J) IL-4. (K) IFN-γ. Data are expressed as the mean ± S.E.M and were analyzed by one-way ANOVA followed by the Tukey's post hoc test. Significance was defined as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, vs. controls groups ($n = 20$ per group).

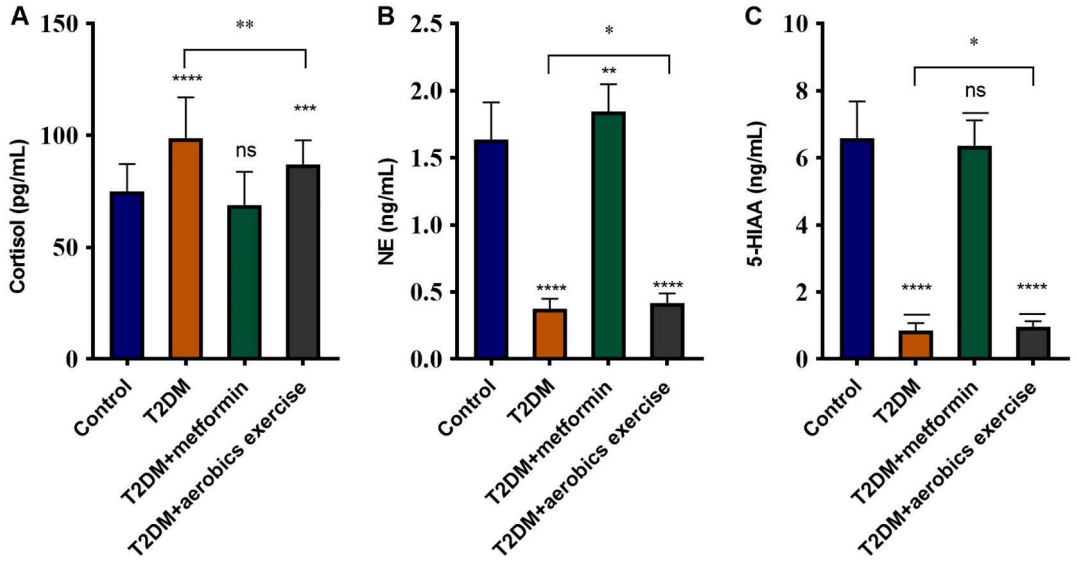

Fig. 4. Depression factors of zebrafish use whole brain tissue examination. (A) Cortisol. Cortisol levels in T2DM + aerobic exercise group were lower than those in the T2DM group ($p = 0.004$). (B) NE. Compare with T2DM group, aerobic exercise promoted NE secretion ($p = 0.0406$). (C) 5-HIAA. Compare with T2DM group, aerobic exercise promoted 5-HIAA secretion ($p = 0.0243$). The data are expressed as the mean ± S.E.M and were analyzed by one-way ANOVA followed by the Tukey's post hoc test. Significance was defined as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, vs. controls groups ($n = 20$ per group).

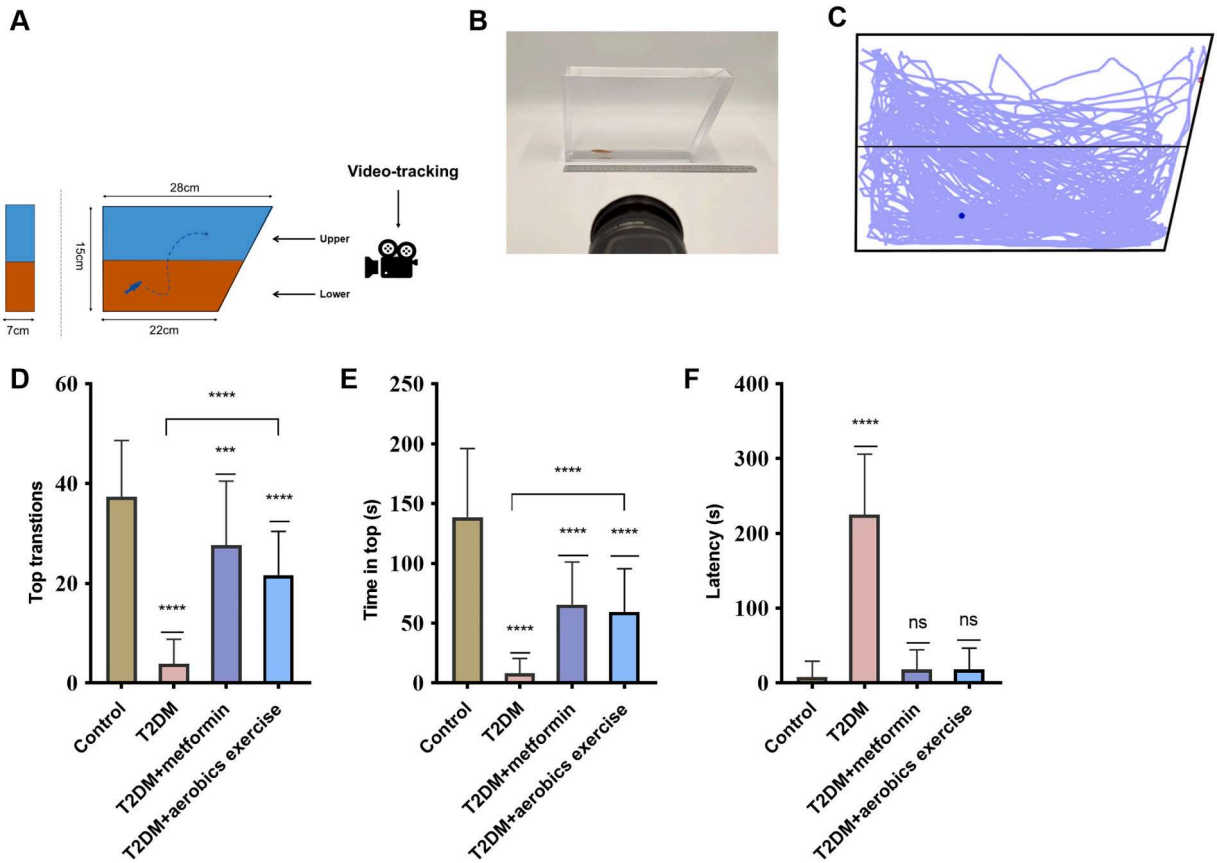

Fig. 5. Depression-like behavior of zebrafish in the novel tank test (NTT). (A) NTT schematic. (B) NTT physical picture display. (C) Zebrafish trajectory diagram in NTT, the blue dots represent the end points of trajectory. (D) The number of top transitions in NTT, transitions number in T2DM + aerobic exercise group was more than that in the T2DM group ($p < 0.0001$). (E) Time for zebrafish to explore at the top in NTT, T2DM + aerobic exercise group showed a longer time ($p < 0.0001$) of exploration compared to the T2DM group. (F) Latency for zebrafish to enter the top. The data are expressed as the mean ± S.E.M and were analyzed by one-way ANOVA followed by the Tukey's post hoc test. Significance was defined as $^{***}p < 0.001$, and $^{****}p < 0.0001$, vs. controls groups ($n = 20$ per group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

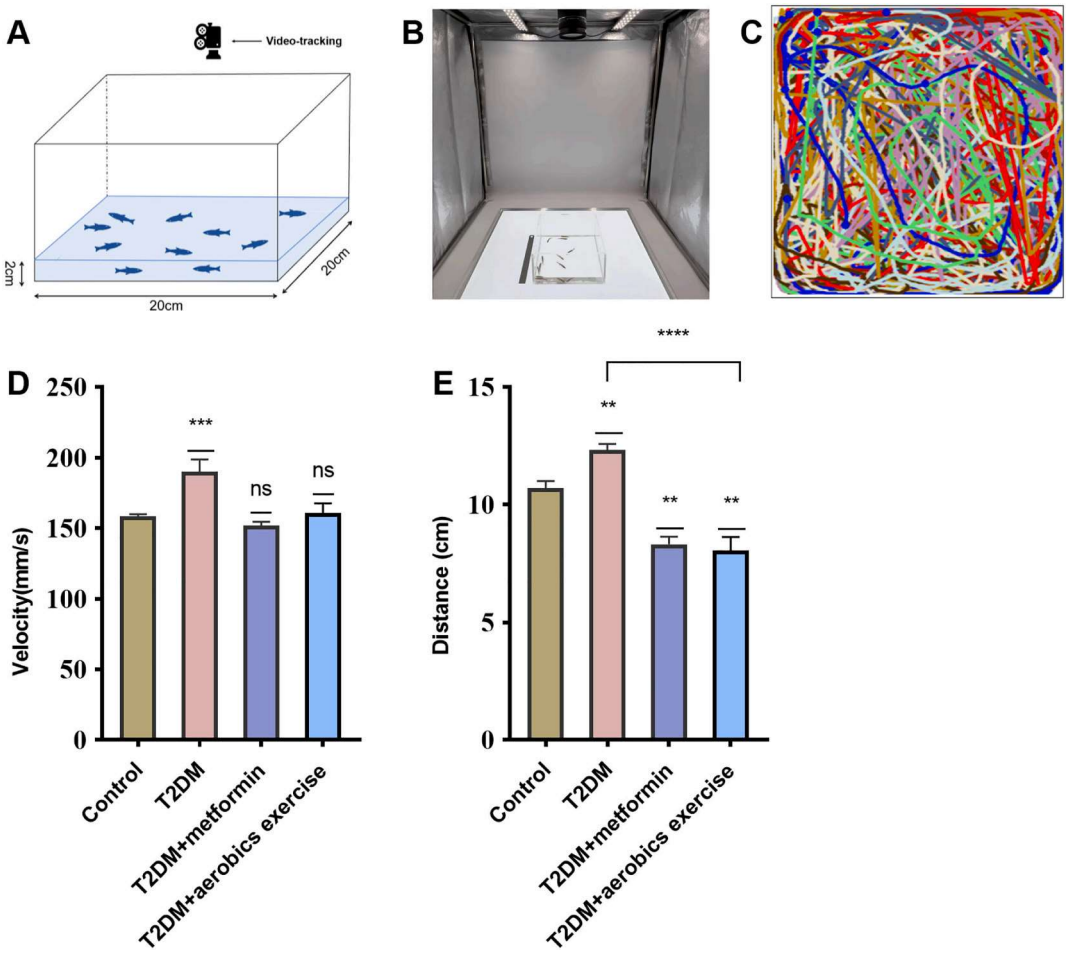

Fig. 6. Depression-like behavior of zebrafish in the shoaling test. (A) Shoaling test schematic. (B) Shoaling test physical picture display. (C) Zebrafish trajectory diagram in shoaling test, 10 zebrafish in each group participated in the shoaling test and the trajectory is represented by different colors. (D) Velocity for zebrafish in shoaling test. (E) Distance between zebrafish in shoaling test, T2DM + aerobic exercise group show a closer distance between zebrafish than that in T2DM group ($p < 0.0001$). The data are expressed as the mean ± S.E.M and were analyzed by one-way ANOVA followed by the Tukey's post hoc test. Significance was defined as $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, vs. controls groups ($n = 20$ per group).

of glucagon (Fig. 7A) more than T2DM + aerobic exercise, suggesting that glucose tolerance may be impaired after metformin intervention in healthy subjects [83]. Additionally, aerobic exercise effectively decreased CD11b (Fig. 7C), IL-6 (Fig. 7D), TrkB (Fig. 7I), and Bcl-2 (Fig. 7K) levels, as well as Bax (Fig. 7M). Moreover, aerobic exercise maintained a greater Bcl-2/Bax ratio (Fig. 7L) relative to the T2DM group, implying a decreased risk of apoptosis which is associated with diabetes [84].

4. Discussion

Herein, a zebrafish model of T2DM complicated with depression was successfully established. As with mammals, over-nutrition results in an increase in blood glucose and BMI levels [85], and induce liver adipose metabolism disorder (Fig. 3) in zebrafish [86,87]. It should be noted that IL-1 (Fig. 3I) mediated IL-1β plays a key role in tissue inflammation caused by metabolic stress [88]. Increased levels of IL-4 (Fig. 3J) suggest activated TH2 cells in T2DM, whereas it reduces 5-HT levels in mental disorders by suppressing tryptophan hydroxylase (TPH) mRNA and activating serotonin transporter (SERT) [89]. Clinically, IFN-γ also induces tryptophan degradation in T2DM patients, which significantly promotes the risk of developing depression (Fig. 3K) [90,91]. Meanwhile, our results demonstrated that this method applies to the zebrafish T2DM depression model and avoids the risk of high mortality associated with glucose-induced susceptibility in previous studies [38]. To address the common cause of T2DM between zebrafish and humans, which is primarily diet, a better approach would be to transform the zebrafish fodder. Although the zebrafish T2DM model (including drug induction) can achieve an effect in a short time [37,92], high glucose fairy shrimp fodder shows safety and efficacy, which provided a novel strategy for establishing zebrafish T2DM model in this study.

T2DM-related metabolic disorders present a chronic inflammatory state, leading to brain damage and the rapid onset of mental disorders such as depression and neurodegenerative diseases [6]. Additionally, diabetes induces oxidative stress in tissues, including peripheral nerve tissue [93]. These findings confirmed the feasibility of the zebrafish diabetes mellitus depression model. Novelty, present study used reserpine to induce depression-like behavior in zebrafish. Based on meeting the levels of diabetes biomarkers, zebrafish showed sustained high levels of cortisol (Fig. 4A) secreted by the HPI axis in zebrafish. The decreased level of NE (Fig. 4B) suggested that NE activated its receptors (α1, α2, β) attempt to improve the mental state [94]. Additionally, the NE system has complex neurotransmission of 5-HT, which results in a decrease in 5-HIAA levels (Fig. 4C) in depression [95]. The presence of elevated depression-like behavior in both the NTT (Fig. 5) and the shoaling test (Fig. 6) confirmed the relationship between T2DM and depression. On the one hand, T2DM-induced neuroinflammation leads to neuronal apoptosis in zebrafish [96]. On the other hand, elevated TG (Fig. 3D) increases its metabolite (cortisol) [97].

This study revealed that aerobic exercise improves depression-like

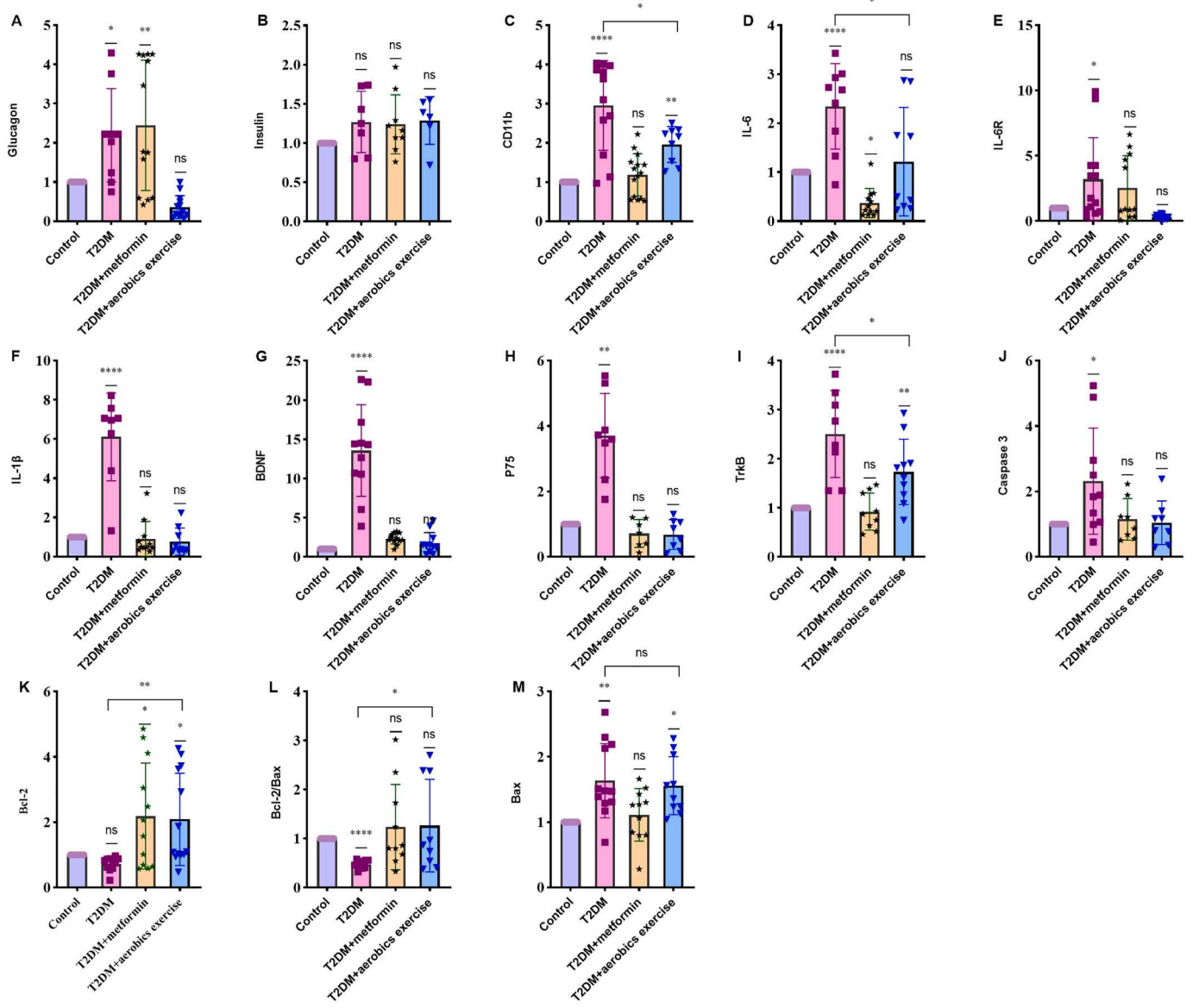

Fig. 7. Gene expression levels among different treatment groups. Groups correspond to different color bars: control group (purple), T2DM group (pink), T2DM + metformin group (yellow), T2DM + aerobic exercise group (blue). (A) Glucagon. (B) Insulin. (C) CD11b, compare with T2DM group, the T2DM + aerobic exercise group shows a lower expression of CD11b ($p = 0.0239$). (D) IL-6, compared to T2DM group, T2DM + aerobic exercise group showed a lower expression of IL-6 ($p = 0.0332$). (E) IL-6R. (F) IL-1β. (G) BDNF. (H) p75. (I) TrkB, compared to T2DM group, T2DM + aerobic exercise group showed a lower expression of TrkB ($p = 0.0493$). (J) Capase 3. (K) Bcl-2, compared to T2DM group, the T2DM + aerobic exercise group showed a lower expression of Bcl-2 ($p = 0.0046$). (L) Bcl-2/Bax, compared to T2DM group, the T2DM + aerobic exercise group showed a lower expression of Bcl-2/Bax ($p = 0.0120$). (M) Bax. The data are expressed as the mean ± S.E.M and were analyzed by one-way ANOVA followed by the Tukey's post hoc test. Significance was defined as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, vs. controls groups ($n = 6$–10 per group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

symptoms in T2DM zebrafish and decreases the risk of diabetes. We constructed an equipment that can be used to carry out multiple experiments of zebrafish aerobic exercise (Fig. 2). Specifically, aerobic exercise decreased high blood glucose, body fat, and inflammation caused by T2DM, and maintained a positive behavior of zebrafish. The duration of aerobic exercise has previously been shown to be proportional to the status of physical health [98]. Previous studies have shown that aerobic exercise can directly or indirectly regulate the activity of islet [41,99] and neurogenesis [100–102].

In this study, aerobic exercise effectively compensated for the impaired signaling of the insulin-glucagon pathway in T2DM (Fig. 7A–B) [11,103]. Unlike metformin (which is affected by insulin sensitivity) [104], exercise maintained normal baseline of insulin. Muscle contraction and exercise activate muscle glucose uptake [99], show a lower blood glucose and BMI (Fig. 3). This study demonstrated that aerobic exercise was more effective in maintaining normal levels of TG (Fig. 3D) and T-CHO (Fig. 3E) compared to metformin. Notably, reduced body fat results in changes to the level of HDL-C and LDL-C [105]. Aerobic exercise significantly decreased LDL-C (Fig. 3F) and increased HDL-C levels (Fig. 3G). Moreover, our research found that metformin increased the levels of TP (Fig. 3H) as did in T2DM zebrafish. However, TP was significantly higher after exercise, suggesting that protein intake should be increased to enhance glycogen storage and prevent muscle injury [106]. Regular exercise indirectly suppresses inflammation and activates IL-1ra [107]. In addition, strenuous exercise has been reported to increase the risk of inflammation mediated by IL-4

and IFN-γ [108]. Surprisingly, the present findings demonstrate that aerobic exercise normalized levels of IL-1, IL-4, and IFN-γ more effectively compared to metformin (Fig. 3).

Aerobic exercise increased the level of 5-HIAA (Fig. 4C) and the effect on depression [109], as well as decrease cortisol levels (Fig. 4A). Furthermore, aerobic exercise increased the level of NE (Fig. 4B), probably due to the promotion of dopamine synthesis in the striatum under the catalysis of dopamine beta hydroxylase [110,111]. Evidence from prior studies has shown that serotonin regulates proliferation and neurogenesis in vivo [112–115]. Compared with T2DM group, aerobic exercise elevated the level of 5-HIAA (Fig. 4C) ($p = 0.0243$) associated with the excitability of CNS. Following that, further experiments were carried out to investigate the effect of aerobic exercise on zebrafish depression-like behavior. Like in humans, the distance between individuals can be a proxy indicator of sociability in zebrafish. Aerobic exercise exhibited higher boldness, exploratory behavior, and tended to socialize more (Fig. 5–6) compared with the T2DM group [116,117].

Aerobic exercise promoted the expression of CD11b (Fig. 7C) via microglia cell damaging factors [118]. Inflammation and high blood glucose have been shown to cause nerve injury [93,119]. However, we found that exercise reduced IL-1β (Fig. 7F), IL-6 (Fig. 7D), and IL-6R (Fig. 7E), demonstrating that aerobic exercise effectively prevents neuro-inflammation. Although aerobic exercise maintained baseline levels of BDNF (Fig. 7G) and P75 (Fig. 7H), it elevated TrkB (Fig. 7I), which demonstrated that aerobic exercise prevents nerve damage through the BDNF-TrkB pathway [120]. Moreover, aerobic exercise suppressed neuronal apoptosis as evidenced by reduced levels of apoptotic genes (caspase-3) (Fig. 7J). Although, it did not reduce expression of apoptosis-regulating gene, Bax (Fig. 7M), it upregulated Bcl-2 expression (Fig. 7K) to decrease mitochondrial permeability [121] and neuronal apoptosis. Results shown in Fig. 7 demonstrate that aerobic exercise improves depression-like symptoms in T2DM zebrafish by affecting apoptosis mechanisms.

5. Conclusions

In general, aerobic exercise has been shown to improve the symptoms of diabetes by directly promoting fat-burning [122]. Strength training is the recommended form of exercise because its effect lasts longer and it improves the body shape. Moreover, aerobic exercise has other benefits such as strengthening of the function of immune system [123]. In addition, prolonged aerobic exercise increases the formation of blood capillaries in skeletal muscle [124,125]. Capillaries promote absorption of nutrients such as protein and carbohydrate [126] to provide energy for muscle activities. It has also been shown that aerobic exercise can increase the number of mitochondria [127], hence improve aerobic metabolism in skeletal muscle. Therefore, incorporation of aerobic exercise and strength training might provide better clinical benefits. Moreover, the above findings demonstrate that the benefits of exercise depend on optimal choice and combination of various types of exercises.

This study, and other previous reports, demonstrated that aerobic exercise has profound benefits on physical and mental health [128,129]. Apart from producing positive emotional feelings, aerobic exercise also promotes good character development. Exercise clears the mind and relaxes body muscles by decreasing stress in nervous system [102].

In summary, the present study developed an improved zebrafish model of T2DM depression, and investigated the effect of aerobic exercise on depression-like behavior in T2DM zebrafish. Collectively, the results demonstrate the link among aerobic exercise, depression, and T2DM-related pathophysiology in zebrafish. Given that the neurochemistry between humans and zebrafish is remarkably similar, this work reveals that aerobic exercise has important clinical significance, especially in the prevention of T2DM complicated with depression.

Abbreviations

T2DM	type 2 diabetes mellitus
CNS	central nervous system
HPI	hypothalamic-pituitary-interrenal
HPA	hypothalamic-pituitary-adrenal
CUMS	chronic unpredictable mild stress
BMI	body-mass index
NTT	novel tank test
TG	triglyceride
T-CHO	total cholesterol
HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
TP	total protein
IL-1	Interleukin-1
NE	norepinephrine
5-HIAA	5-hydroxyindole acetic acid
BDNF	Brain-derived neurotrophic factor
TrkB	tyrosine kinase receptor B
P75	P75 neurotrophin receptor
Bcl-2	B-cell lymphoma-2

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CRediT authorship contribution statement

Lei Wang: Visualization, Investigation. **Jiahui Ma:** Writing – original draft. **Wei Wu:** Writing – review & editing. **Yimeng Fang:** Data curation. **Fan Liu:** Investigation. **Qinsi Yang:** Visualization. **Xiang Hu:** Software. **Xuejiang Gu:** Software. **Zhiying He:** Validation. **Da Sun:** Conceptualization, Methodology. **Libo Jin:** Supervision. **Xingxing Zhang:** Conceptualization, Methodology.

Declaration of competing interest

The authors declare that they have no competing interests.

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