

Research Article

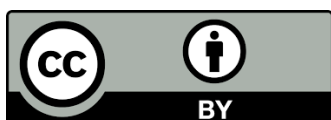
Moderate Exercise Suppresses Tumor Growth and Progression through Regulating CytokinesJunfeng Zhang ^{1,†}, Yajie Li ^{2,†}, Qianghua Xue ², Wen He ², Chenchen Li ^{3,*}, Yanli Wang ^{3,*}

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* **Correspondences:** Chenchen Li and Yanli Wang; E-Mails: lichenchen0128@163.com; wangyanli@staff.shu.edu.cn;**Academic Editor:** Pedro Morouco**Special Issue:** [The Impacts of Physical Activity, Exercise, and Sports on Human Health](#)*OBM Integrative and Complementary Medicine*
2025, volume 10, issue 2
doi:10.21926/obm.icm.2502021**Received:** January 21, 2025**Accepted:** April 26, 2025**Published:** May 12, 2025**Abstract**

Evidence suggests that regular moderate exercise can improve symptoms of depression and may enhance immune function, with an interesting role in cancer prevention and improved cancer-related prognosis; however, over-exercising may lead to overtraining and generate psychological symptoms that mimic depression, and may impair immune function. In this



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study, we chose swimming as an exercise form to conduct research. Swimming groups were divided into 5, 20, and 60 min day⁻¹, and open field tests (OFT) were used to assess the mice's behavior in desperation. The results showed that regular moderate swimming (5 min day⁻¹, 62 days) inhibited tumor development and progression by regulating the expression levels of cytokines (IL-6, TNF- α , and IFN- γ) involved in cellular immune responses. However, the overload swimming (20 and 60 min day⁻¹, 62 days) caused depression in mice and promoted tumor growth and progression. In addition, moderate swimming also prolonged the survival of tumor-bearing mice. Our findings prove that moderate exercise can improve immunity and protect against cancer. In contrast, overload exercise can cause psychological stress such as depression, as well as guide the development of proposals during cancer treatment and postoperative recovery.

Keywords

Moderate exercise; immune cytokine; swimming; depression; tumor

1. Introduction

Adherence to regular exercise has been associated with significant health benefits for healthy individuals and those with chronic conditions [1]. There is a clear interest in identifying the likely role of exercise in cancer prevention and improving cancer-related prognosis. Research has demonstrated that exercise can inhibit tumor initiation and progression, and it is also linked to improved health status and quality of life [2-8]. Recent studies have highlighted the antitumor effects of exercise in various cancers through specific receptors on tumor cells and epithelial cells, primarily involving immune factors. Vulczak *et al.* have shown that moderate exercise regulates tumor metabolism in triple-negative breast cancer, thereby inhibiting tumor growth [9]. Luo *et al.* showed that physical exercise reverses the immunocold tumor microenvironment via inhibiting SQLE in non-small cell lung cancer [10]. Zarei *et al.* showed that the exercise-inducing myokine irisin significantly mitigated the proliferation, migration, and invasion of ovarian cancer cells via the HIF-1 α signaling pathway [11]. Wan *et al.* showed that exercise potentially prevents colorectal cancer liver metastases by suppressing tumor epithelial cell stemness via RPS4X downregulation [12]. Orange *et al.* showed that acute aerobic exercise-conditioned serum reduces colon cancer cell proliferation in vitro through interleukin-6-induced regulation of DNA damage [13]. Epidemiological studies suggest that consistent physical activity and structured exercise routines can enhance immune function, regulate immune responses, and decrease the risk of numerous chronic diseases. These include infectious diseases such as viral and bacterial infections, cancer, chronic inflammatory conditions, and other non-communicable diseases [14-16].

In addition, studies have shown that regular exercise can improve symptoms of depression, anxiety and panic, with beneficial effects that appear to be equivalent to meditation or relaxation and may enhance immune function [17]; however, overloaded exercise may lead to overtraining and generate psychological symptoms that mimic depression, and may impair immune function [18, 19]. Until now, the mechanism of the influence of moderate exercise and overload exercise on the occurrence and development of cancer is not clear. In here, our findings provide strong evidence

that moderate exercise improves immunity and protects against cancer, while overloading exercise can cause psychological stress, such as depression, as well as provide guidance for the development of proposals during cancer treatment and postoperative recovery.

2. Materials and Methods

2.1 Materials

Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were obtained from YoBiBiotech Co., Ltd. (Shanghai, China). D-Hank's buffer was prepared in our laboratory. A 25% trypsin-EDTA solution was purchased from Gino Biological Pharmaceutical Technology Co., Ltd. (Hangzhou, China). Formalin solution (4% v/v) was provided by Daan Gene Co., Ltd. (Shanghai, China).

2.2 Cell Culture

The B16-F10 mouse melanoma cell line was obtained from the Chinese Academy of Sciences. Cells were cultured in high-glucose DMEM supplemented with 10% FBS and 5% penicillin-streptomycin at 37°C in a 5% CO₂ and 95% air atmosphere.

2.3 Animal Studies and Swimming Grouping Based on Duration

Female C57BL/6 mice (20 ± 2 g; 6-7 weeks old) were sourced from Shanghai Jiesijie Experimental Animal Co., Ltd. (Shanghai, China). The mice were housed in a controlled environment with plastic cages, maintaining 55% humidity, a 12-hour light/dark cycle, and a temperature of 22 ± 0.5°C [20]. They were acclimated for one week before the experiments. Swimming is commonly used as a form of exercise in rodent disease models [21], as it helps prevent stress-related injuries that can occur with treadmill running [22]. In the pilot study, C57BL/6 mice were subjected to daily swimming sessions in water maintained at 30 ± 2°C for 62 days.

The mice were divided into four groups: (1) Control group—mice did not swim and were fed typically; (2) 5 min day⁻¹—mice swam voluntarily for 5 minutes; (3) 20 min day⁻¹—after the initial voluntary swimming, mice were encouraged to continue swimming for a total of 20 minutes; and (4) 60 min day⁻¹—mice were forced to swim for 60 minutes. All mice swam for 5 minutes during the first week for adaptation. The swimming duration gradually increased from 20 minutes in the second week to 60 minutes in the third week, with the training period maintained at 60 minutes for the remainder of the study [7]. Group training was used, as it promotes more intense physical activity compared to individual swimming sessions [23]. Initially, the mice swam voluntarily for approximately 5 minutes before floating and swimming intermittently. To encourage continuous swimming for longer durations, a stick was used to prod them to keep moving [24] gently. After 39 days, all C57BL/6 mice were injected with B16-F10 cells (1 × 10⁵ or 2 × 10⁵ cells/mL) subcutaneously in the right abdomen to induce tumorigenesis. Then each group of mice continued the experiment for 23 days according to the original plan. Animals were manually palpated three times a week, and the day of tumor detection was recorded (latency to approximately 50 mm³). The tumor size was measured with a caliper three times a week, and body weight was recorded every two days. Tumor volume was calculated using the formula: $V_{\text{tumor}} = L \times W^2/2$ (where L is tumor length and W is tumor width) [25]. At the end of the experiment, we sacrificed the mice by dislocating their joints.

2.4 Depression Model

The psychological intervention took the form of acute restraint stress, in which the mice were placed in a space that restricted their free movement but did not compress them excessively [26]. This experiment used a 50 mL centrifuge tube to bind the mice for 2 hours a day [27]. The holes along the side wall of the tube were used to circulate the air and ensure the mice were breathing normally. Control mice were placed in cages and fed typically. After 7 days, all C57BL/6 mice were injected with B16-F10 cells (1×10^5 or 2×10^5 cells/mL) subcutaneously in the right abdomen to induce tumorigenesis. Then each group of mice continued the experiment for 21 days according to the original plan.

2.5 Open Field Test (OFT)

The open-field test was conducted in a rectangular plastic chamber (50 cm × 50 cm × 50 cm), with the floor divided into 16 equal squares. The central four squares were designated as the "central area," and the remaining squares were categorized as the "peripheral area." Before the test, the mice were given an appropriate time to adapt to the environment of the test room. The room should be quiet and dark during the test, and the site should be cleaned with 70% ethanol at the end of each experiment. To avoid contamination from previous animals, the chamber was cleaned and allowed to dry before each new test. During the test, mice were placed in the center of the open field and allowed to explore freely for 5 minutes. Cameras were used to track each animal's movements, infrared laser trackers and sensors to identify the central point of the mouse's body, and behavior was recorded using analysis software (Stoelting Any-maze). All the mice continued to swim for 62 days, and we carried out the OFT.

2.6 Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of IL-6, TNF- α , and IFN- γ were measured using specific ELISA kits for mouse IL-6, TNF- α , and IFN- γ by Shanghai Hu Ding Biological Technology Co., Ltd. (Shanghai, China). The procedures for performing the enzyme-linked immunosorbent assays followed the manufacturer's instructions.

2.7 Evaluation of Toxicity in Vivo

Organs were carefully dissected, weighed, and immediately frozen. Samples were stored at -80°C until further analysis. Serum was collected by centrifuging blood samples at 3,000 rpm for 10 minutes. To assess liver function, the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were measured. An elevation in these enzyme levels typically indicates liver dysfunction. Kidney function was evaluated by measuring the levels of urea nitrogen (UREA) and creatinine (CRE), with increases in these parameters suggesting nephrotoxicity. All biochemical measurements were performed using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) following standard protocols outlined in the literature.

Histopathological examination was carried out using conventional methods. Tissues from the heart, liver, spleen, lungs, kidneys, and brain were excised, fixed in 4% (v/v) formalin solution, and embedded in paraffin. Sections were cut to a thickness of 5 μ m and placed onto glass slides. After Hematoxylin and Eosin (H&E) staining, the slides were analyzed by a pathologist using an optical

microscope (Nikon U-III Multi Point Sensor System, USA).

2.8 Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8.1 (GraphPad, La Jolla, CA, USA). Mice that died due to drowning were excluded from the survival analysis. Continuous variables were compared using two-tailed t-tests (for comparisons between two groups) or one-way analysis of variance (ANOVA) (for comparisons among multiple groups), assuming normal distribution. The homogeneity of variances was also tested. A p-value of less than 0.05 was considered statistically significant. All charts and graphs were created using GraphPad Prism 8.1.

2.9 Ethical Statement

All animal procedures were conducted in accordance with "The National Regulation of China for Care and Use of Laboratory Animals" and were approved by the Institutional Animal Care and Use Committee of Shanghai University (Approval No. ECSHU2021-109).

3. Results

The results showed that regular moderate swimming inhibited tumor development and progression by regulating the expression levels of cytokines (IL-6, TNF- α and IFN- γ) involved in cellular immune responses. However, the overload swimming caused depression in mice and promoted tumor growth and progression.

3.1 Moderate Swimming Depressed Tumor Growth and Progression

After 39 days of continuous swimming in mice, tumor models carrying B16-F10 cells were established. Tumor size was measured thrice a week, and all mice were sacrificed 23 days post-tumor inoculation. Tumor volume and weight measurements (Figure 1a, b) indicated that moderate swimming (5 min day⁻¹, 62 days) significantly reduced tumor growth compared to the control (Ctrl) group. In contrast, overload swimming (60 min day⁻¹, 62 days) enhanced tumor growth, with the 60-minute group showing a significantly greater effect than the control group ($P = 0.0045$). Additionally, we calculated the percentage of mice with tumor volumes greater than or equal to 100 mm³ at different time points (Figure 1c). The incidence of tumor formation was significantly higher in the overload swimming groups than in the moderate swimming group, with the overload groups exhibiting earlier tumor onset. To further assess the impact of swimming, survival curves were analyzed, and the results showed that regular moderate swimming (5 min day⁻¹, 62 days) prolonged survival, whereas overload swimming (20 and 60 min day⁻¹, 62 days) shortened survival time (Figure 1d).

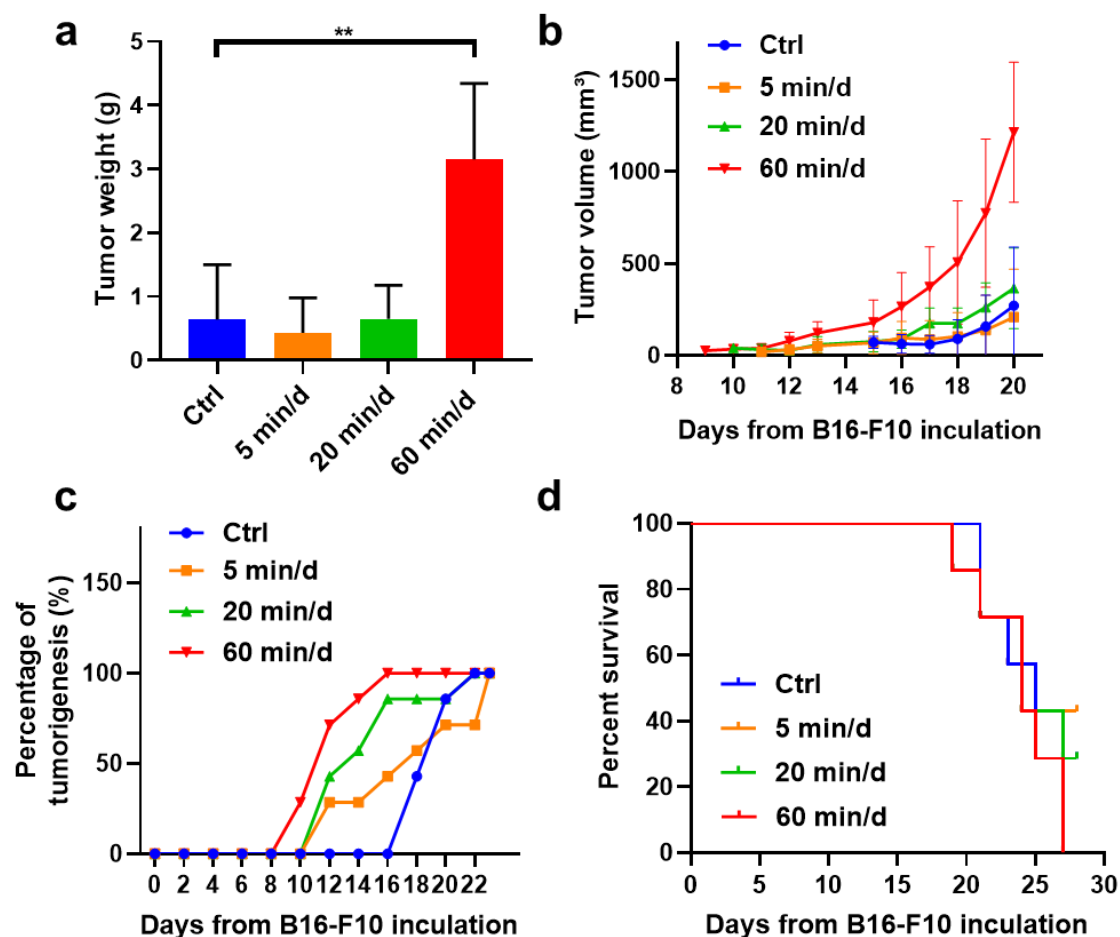


Figure 1 Moderate exercise depressed tumor growth and progression. (a) The tumor weight was measured on the day of sampling in the four groups (0, 5, 20, and 60 min of day⁻¹ swimming exercise). (b) Tumor volume measurements were taken every 2 days after tumor cell injection in different experimental groups (n = 6). (c) The percentage of mice with tumorigenesis is the tumor volume reaching 50-100 mm³. (d) Log-rank survival analysis of the four groups. Data are presented as mean ± SD (P values between indicated groups), **p < 0.01.

3.2 Overload Swimming Causes Depression in Mice

Mice in all experimental groups had no significant difference in body weight throughout the experiment (Figure 2a). Behavioral despair of mice was assessed using an open field test (OFT), and two-tailed t-test results showed that mice with overload swimming had reduced total distance traveled, time of center stay, and times of center entries compared to the control group. These differences were statistically significant (Figure 2b-h). This indicates that the mice in the overload swimming group exhibited depressive behaviors after swimming for 62 days.

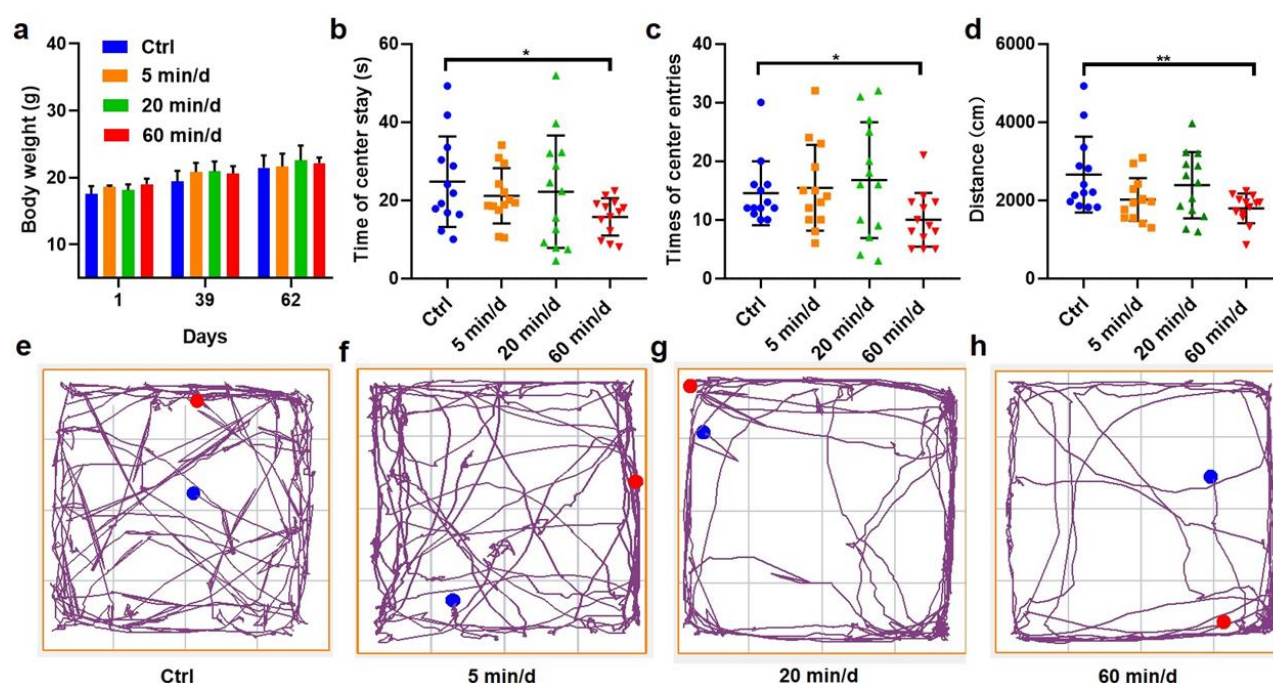


Figure 2 Overload swimming causes depression in mice. (a) Body weight measurements were taken on days 1, 39, and 62 for the four groups (0, 5, 20 and 60 min day⁻¹ swimming exercise) (n = 13). (b–d) The Open-Field Test (OFT) assessed behavioral changes following inoculation of 2×10^5 B16-F10 cells in different experimental groups. (b) Time of center stay in the OFT. (c) Times of center entries in the OFT. (d) Total distance in the OFT. (e–h) Representative trajectory maps showing the autonomous activity of mice in the OFT after 2×10^5 B16-F10 cell inoculation. Statistical analysis was performed using two-tailed t-tests for pairwise comparisons. The results were expressed as the mean $\pm$ SD (P values are shown between indicated groups), *p < 0.05, **p < 0.01.

To investigate whether overload swimming induces depression, we employed the acute restraint (AR) stress procedure to establish a depression model (Figure 3a) [25]. The results showed that C57BL/6 mice subjected to AR stress exhibited signs of depression after 2 weeks. Mice in the AR group showed a slower increase in body weight compared to the control group, with a significant difference observed (Figure 3b). Additionally, behavior was assessed using the Open-Field Test (OFT). The AR group displayed reduced total distance traveled and less time spent in the center than the control group, with statistical significance (Figure 3c, d). These findings further support the conclusion that overload swimming induces depressive-like behavior in mice.

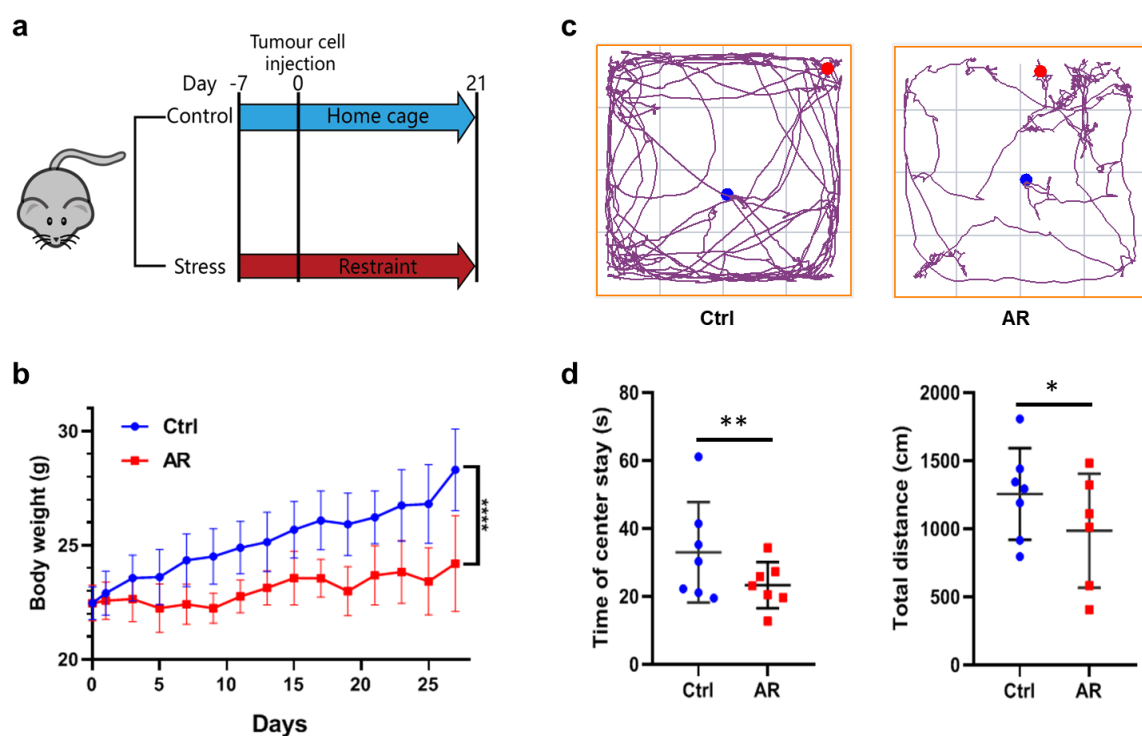


Figure 3 Establishment of the acute restraint (AR) stress-induced depressed mice models. (a) Schematic diagram of the experimental design for the depression model. (b) The body weight was measured every 2 days until sacrifice. (c) Typical trajectory diagram of the autonomous activity in the OFT. (d) Time spent in the central area and total distance traveled in the OFT. The results were expressed as the mean $\pm$ SD (P values are shown between indicated groups), *p < 0.05, **p < 0.01, ****p < 0.0001.

3.3 Depression Promotes Tumor Growth and Progression

After 2 weeks of acute restraint (AR) stress, mice with the B16-F10 tumor model were established. Tumor size was measured daily, and all mice were sacrificed 21 days post-tumor induction. Our findings showed that depression accelerated tumor growth, with no difference in tumor volume (Figure 4a) but a significant difference in tumor weight (P = 0.0361) compared to the control group (Figure 4b). Additionally, we calculated the percentage of mice with tumors reaching a volume of 100 mm³ or greater at various time points. The incidence of tumorigenesis in the AR stress group was notably higher than in the non-stressed group (Figure 4c). Survival analysis further revealed that depression induced by AR stress shortened the lifespan of the mice (Figure 4d). These results closely mirrored the effects observed in the overload swimming groups (20 and 60 min day⁻¹, 62 days), suggesting that depression accelerates tumor growth and progression.

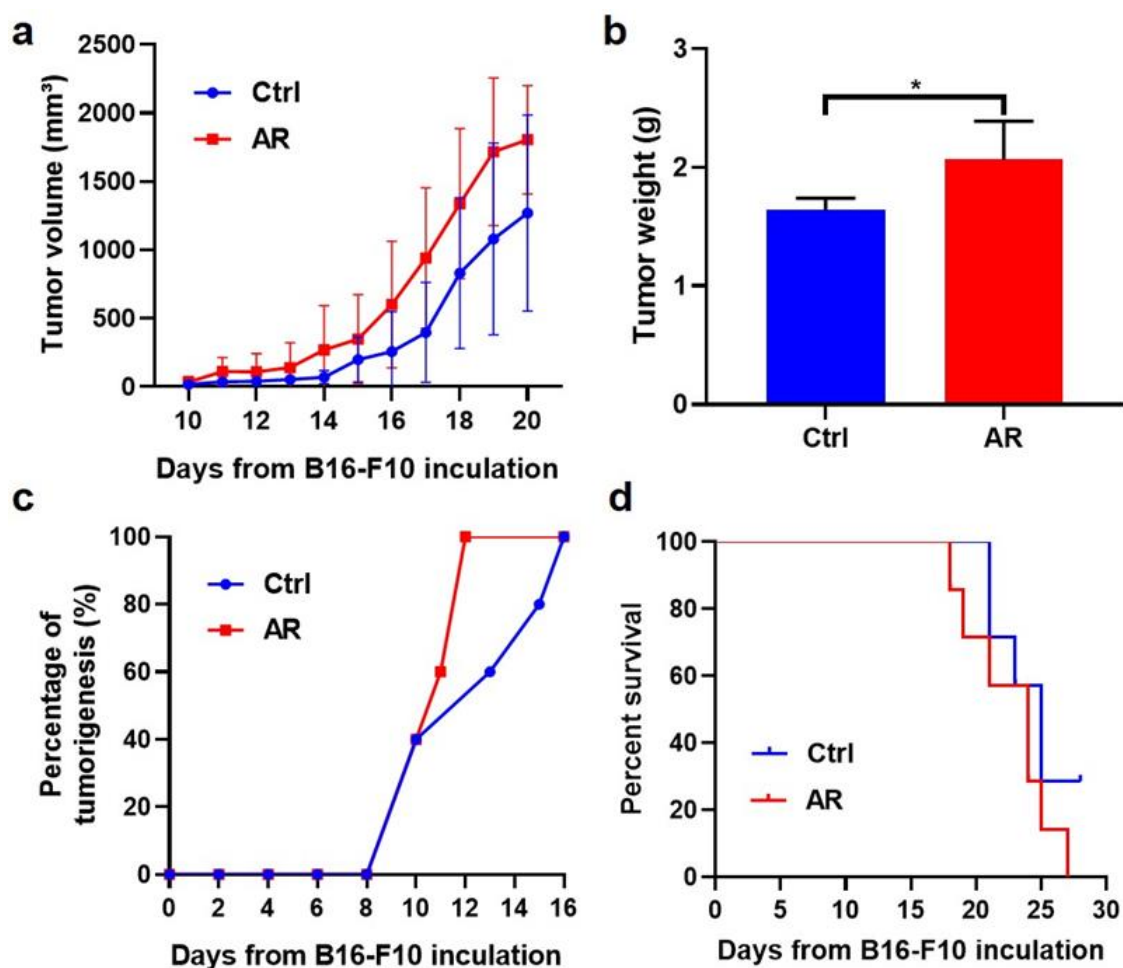


Figure 4 Depression promoted tumor growth and progression in mice. (a) The tumor volumes were measured every 2 days after tumor cell injection ($n = 6$). (b) Tumor weight was recorded at the time of sampling. (c) The proportion of mice with tumor formation was calculated. Tumorigenesis was defined as the tumor volume reaching 50-100 mm³. (d) Log-rank survival analysis of the two groups. Data are presented as mean $\pm$ SD (P values between indicated groups), * $p < 0.05$.

3.4 Moderate Swimming Increases Immune Cytokine Levels

Pro-inflammatory cytokines are small proteins crucial in regulating the immune system to combat cancer [28]. IL-6, TNF- α and IFN- γ are typical pro-inflammatory cytokines in cellular immune response [29-31].

We analyzed the levels of IL-6, TNF- α and IFN- γ in tumor tissue by ELISA (Figure 5a). The results showed that compared with the control group, the levels of IL-6, TNF- α and IFN- γ in the 5 min day⁻¹ swimming group were significantly increased ($P = 0.0344$, 0.0003 , 0.0001). However, the level of IL-6 in the 20 min day⁻¹ ($P = 0.8827$) and the 60 min day⁻¹ swimming group ($P = 0.1824$) did not change, but the level of TNF- α decreased significantly ($P = 0.0315$, 0.0167). In addition, the level of IFN- γ increased slightly in the 20 min day⁻¹ swimming group ($P = 0.0008$) and decreased in the 60 min day⁻¹ swimming group ($P = 0.0131$).

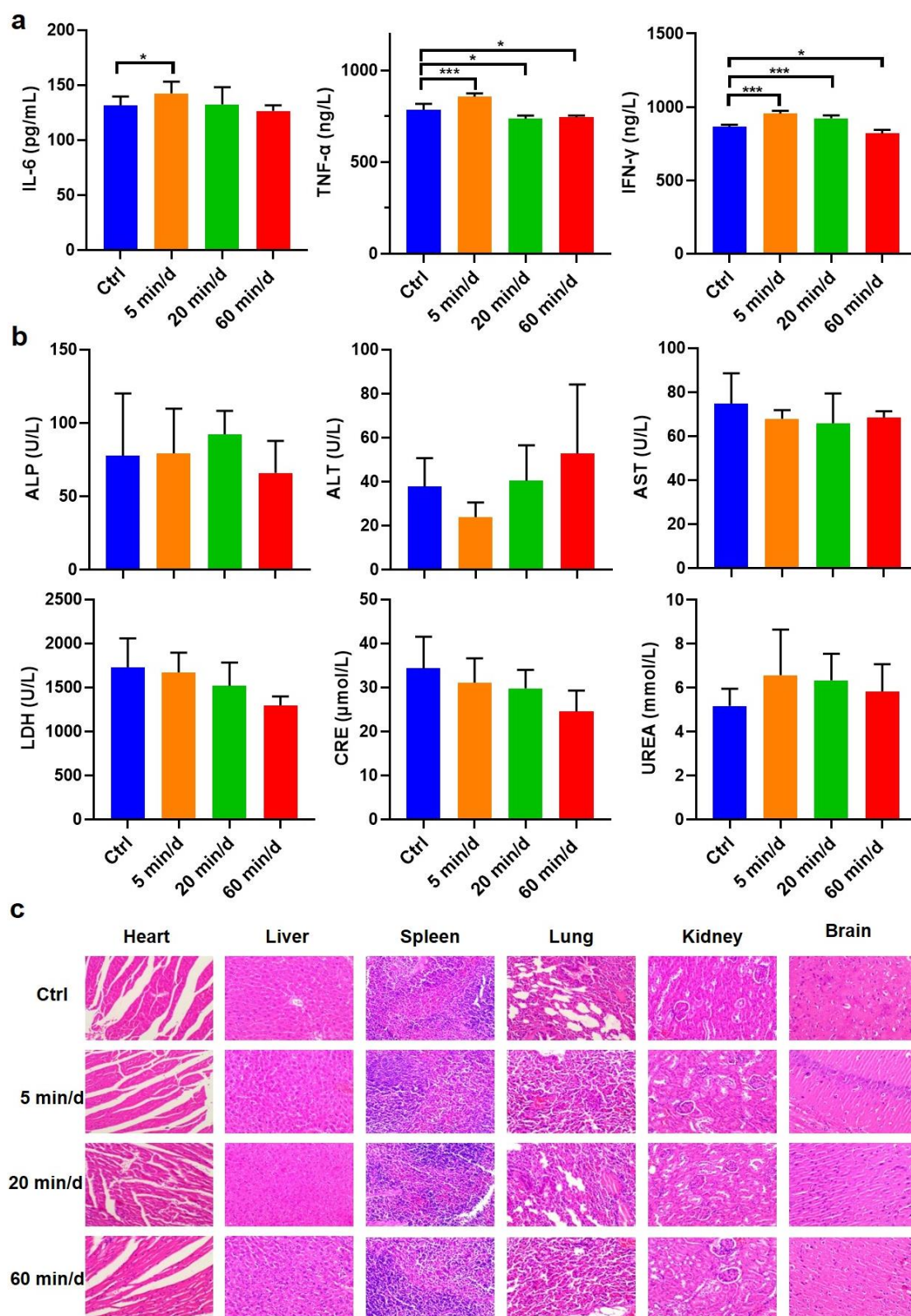


Figure 5 Immunocytokine levels, *in vivo* biosafety and toxicity studies in moderate swimming. (a) The levels of IL-6, TNF-α and IFN-γ in tumor tissue by ELISA. (b) Effects of swimming on serum biochemical parameters: ALP, ALT, AST, LDH, CRE, and UREA. (c) Histological analysis was done using H&E stains of major organs. The results were expressed as the mean ± SD (P values are shown between indicated groups), *p < 0.05, **p < 0.01, ***p < 0.001.

3.5 Biosafety and Toxicity in Vivo

In vivo toxicity was assessed by 6 serum biochemical parameters: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactic dehydrogenase, urea nitrogen, and serum creatinine. The biochemical analysis revealed that these parameters were within normal ranges compared to the control group, suggesting that swimming had no adverse effects on liver or kidney function (Figure 5b).

Histological examination of the major organs further supported the safety findings (Figure 5c). No significant signs of necrosis were observed in key organs such as the heart, liver, spleen, lungs, kidneys, and brain. H&E staining showed that tumor-bearing mice exhibited no infiltrative or degenerative lesions. Compared with the control group, there were no effects on the typical architecture of mice organs in the regular moderate swimming (5 min day⁻¹, 62 days) and overload swimming (20 and 60 min day⁻¹, 62 days).

4. Discussion

Exercise has a direct impact on the cellular immune system, with cytotoxic immune cells, such as NK and T cells, being mobilized into the bloodstream due to stress-induced shear forces and adrenergic signaling during physical activity [32]. Additionally, exercise can indirectly influence immune cell function by releasing immune-modulating cytokines, known as myokines, triggered by muscle contractions [33]. Recent studies have shown that IL-6 promotes immune cell infiltration into tumors during exercise. Moreover, the exercise-induced tumor growth inhibition was significantly reduced when mice were treated with anti-IL-6 antibodies during the exercise regimen [34]. Regular exercise improves depression, anxiety, and panic symptoms, and the beneficial effects appear to be equivalent to meditation or relaxation. However, excessive exercise may lead to overtraining and generate psychological symptoms that mimic depression [18]. In addition, studies have shown that engaging in moderate-intensity exercise may enhance immune function, whereas high-intensity exercise may impair immune function [19]. Our results also show that regular moderate swimming (5 min day⁻¹, 62 days) can significantly increase the levels of IL-6, TNF- α and IFN- γ that regulate cytokines involved in cellular immune responses in mice, and inhibit tumor progression. While overload swimming (20 and 60 min day⁻¹, 62 days) can induce depressive behavior and promote tumor growth.

To confirm that different swimming intensities mainly caused these differences, we subcutaneously injected C57BL/6 mice with murine cancer cells (B16-F10, 1 or 2 $\times 10^5$ mL⁻¹ cells) for 60 min of daily swimming. Behavioral despair of mice was assessed using an open field test (OFT), and two-tailed t-test results showed that mice with overload swimming had reduced total distance traveled, time of center stay, and times of center entries compared to the control group. These differences were statistically significant (Figure S1, Figure S2). This indicates that the mice in the overload swimming group exhibited depressive behaviors. Many researchers have investigated excessive exercise's physiological and psychological implications, concluding that overtraining likely represents a psychobiological disorder [35]. Physiologically, overtraining has been associated with hypercortisolaemia and a hypothalamic dysfunction in response to insulin-induced hypoglycaemia [36]. Psychologically, overtraining can produce a constellation of symptoms, from decreased libido to psychomotor retardation, which mimics depression [18]. Furthermore, we found that tumor volume and weight was increased and significantly different in overloaded swimming mice

compared to the control group. At the same time, the incidence of tumors in the overload swimming group were considerably higher than that in the control group, and the survival time of the mice was shortened significantly. These results all indicate that overload swimming can lead to depression in mice, which in turn promotes the development of tumors (Figure S3). Zhang *et al.* showed that regular moderate swimming (8 min/day, 9 weeks) raised dopamine (DA) levels in the prefrontal cortex, serum and tumor tissue, suppressed growth, reduced lung metastasis of transplanted liver cancer, and prolonged survival in a C57BL/6 mouse model, while overload swimming (16 and 32 min/day, 9 weeks) had the opposite effect [24]. The ELISA analysis confirmed that the levels of IL-6, TNF- α and IFN- γ in the tumor tissue of the mice in the overload swimming group were significantly increased compared with the control group (Figure S4). Serum biochemical parameters showed no apparent changes in experimental mice compared with the control group (Figure S5). Although the weight of the mice did not differ significantly throughout the experiment, the weight gain of the overloaded swimming group was slower than that of the control group (Figure S6). We hypothesized that overloaded swimming affected food intake and nutrition in mice, which needs to be explored in our follow-up experiments.

Our findings demonstrate that moderate exercise enhances immune function and prevents cancer, whereas excessive physical activity induces psychological stress e.g., depression and facilitates cancer progression. Although forced swimming tests are known to trigger stress responses, this particular factor was not addressed in our current experimental design. Future studies will employ controlled exercise paradigms to eliminate confounding stress effects while systematically investigating the molecular mechanisms underlying exercise-mediated regulation of cancer initiation and progression.

5. Conclusion

Exercise is an excellent way to enhance physical fitness and improve human immunity. Theoretically, the expansion effect of the body's immune system can affect the body's resistance to certain diseases, including defenses against tumors. In this study, we chose swimming as an exercise form to conduct research. The results showed that regular moderate swimming (5 min day⁻¹, 62 days) inhibited tumor development and progression by regulating the expression levels of cytokines (IL-6, TNF- α and IFN- γ) involved in cellular immune responses. However, the overload swimming (20 and 60 min day⁻¹, 62 days) caused depression in mice and promoted tumor growth and progression. In here, our findings provide strong evidence that moderate exercise improves immunity and protects against cancer, while overloading exercise can cause psychological stress, such as depression, as well as provide guidance for the development of proposals during cancer treatment and postoperative recovery.

Author Contributions

CL and YW conceived and designed this study. JZ and YL wrote the manuscript. QX and WH participated in the review and revision of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the National Natural Science Foundation of China (82325030 and 52371250) and Science and Technology special fund of Hainan Province (ZDYF2024SHFZ080), National Key Research and Development Program of China (2022YFC2305000), 'Nanhai Xinxing' Science and Technology Innovation Talent Platform Project of Hainan Province (NHXXRCXM202318), and Scientific Research Foundation for High-level Talents of Anhui University of Science and Technology (2024yjrc92).

Ethical Statement

All animal procedures were conducted in accordance with "The National Regulation of China for Care and Use of Laboratory Animals" and were approved by the Institutional Animal Care and Use Committee of Shanghai University (Approval No. ECSHU2021-109).

Competing Interests

There are no conflicts of interest in this article.

Data Availability Statement

The original contributions presented in the study are included in the articles, further inquiries can be directed to the corresponding author.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Figure S1: (A) The body weight was measured on 1 d, 39 d, and 62 d. (B-D) Open-field test (OFT) was applied for statistical analysis on 2×10^5 /mL B16-F10 inoculation. (B) Central area time in the OFT. (C) Times of center entries in the OFT. (D) Total distance in the OFT. (E-F) Typical trajectory diagram of the autonomous activity in the OFT after 2×10^5 /mL B16-F10 inoculation. OFT was based on Two-tailed t-tests for single comparisons. The results were expressed as the mean $\pm$ SD (P values are shown between indicated groups), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
2. Figure S2: (A) The body weight was measured on 1 d, 39 d, and 62 d. (B-D) Open-field test (OFT) was applied for statistical analysis on 1×10^5 /mL B16-F10 inoculation. (B) Central area time in the OFT. (C) Times of center entries in the OFT. (D) Total distance in the OFT. (E-F) Typical trajectory diagram of the autonomous activity in the OFT after 1×10^5 /mL B16-F10 inoculation. OFT was based on Two-tailed t-tests for single comparisons. The results were expressed as the mean $\pm$ SD (P values are shown between indicated groups), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
3. Figure S3: Depression promoted B16-F10 growth and progression in C57 BL/6 mice. (A-D) Subcutaneous 2×10^5 /mL B16-F10 tumors in C57 BL/6 mice ($n = 12$). (E-H) Subcutaneous 1×10^5 /mL B16-F10 tumors in C57 BL/6 mice ($n = 6$). (A, E) The tumor volumes were measured every 2 days after tumor cell injection. (B, F) The tumor weight was measured on the day of

sampling. (C, G) The percent survival was collected from the control and experimental mice. (D, H) The percentage of mice with tumorigenesis was calculated. Tumorigenesis assay was defined as tumor volume reaching 50-100 mm³. The results were expressed as the mean ± SD (P values are shown between indicated groups), *p < 0.05, **p < 0.01, ***p < 0.001.

4. Figure S4: Relative protein levels of IL-6, IFN-γ, and TNF-α in tumor tissue specimens. (A-C) Subcutaneous 2 × 10⁵/mL B16-F10 tumors in C57 BL/6 mice (n = 12). (D-F) Subcutaneous 1 × 10⁵/mL B16-F10 tumors in C57 BL/6 mice (n = 6).
5. Figure S5: Effects of swimming on serum biochemical parameters: ALP, ALT, AST, LDH, CRE, and UREA. (A) Subcutaneous 2 × 10⁵/mL B16-F10 tumors in C57 BL/6 mice (n = 12). (B) Subcutaneous 1 × 10⁵/mL B16-F10 tumors in C57 BL/6 mice (n = 6).
6. Figure S6: Weight gain in the B16-F10 injected Ctrl and swimming 60 min/d mice. (A-C) Subcutaneous 2 × 10⁵/mL B16-F10 tumors in C57 BL/6 mice (n = 12). (D-F) Subcutaneous 1 × 10⁵/mL B16-F10 tumors in C57 BL/6 mice (n = 6). (A, D) Weight gain at B16-F10 injected. (B, E) Weight gain (the whole of the experiment). (C, F) Weight gain in the B16-F10 injected.

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