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Effect of Aerobic Exercise on Serum Metabolites in Mice with Hepatocellular Carcinoma After Surgery

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Statistical Analysis C
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Background: Modern medicine has suggested exercise therapy is one of the main treatments for postoperative rehabilitation of tumors. It can influence the recovery of cancer patients by changing the body's material metabolism and energy metabolism. However, studies on metabolic changes of exercise therapy on hepatocellular carcinoma (HCC) patients after surgery are limited. The aim of this study was to explore the effect of aerobic exercise on mice after orthotopic HCC surgery by serum metabolomics test and explore the related mechanism.

Material/Methods: A total of 60 C57Bl/6 mice were used to establish an orthotopic xenograft model of H22 mouse hepatoma cells. Mice were randomly divided into 6 groups and it was found that the metabolic products of the early postoperative exercise group and sedentary group mainly included L-tryptophan, citric acid, and other energy-related metabolites.

Results: Energy metabolites, such as succinic acid of the high-intensity exercise group were increased after surgery, whereas phospholipid metabolites, including phosphatidylethanolamine (18: 0/0: 0), were decreased. In the moderate-intensity exercise group, the change tendency was consistent, and the level of various metabolites decreased.

Conclusions: Thus, it is likely that aerobic exercise reduced the degree of postoperative stress responses and improved energy metabolism in mice. The underlying mechanism involves improving the tricarboxylic acid cycle, intervening in energy metabolism, reorganization caused by the tumor, reducing the abnormal increase of phospholipase activity caused by the stress of liver cancer, reducing the level of hemolytic phospholipids, thereby inhibiting mitochondrial pathway-initiated apoptosis.

MeSH Keywords: Carcinoma, Hepatocellular • Exercise • Metabolomics • Rehabilitation

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Background

Liver cancer is predicted to be the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide in 2018, with about 841 000 new cases and 782 000 deaths annually [1]. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer with high incidence and mortality, accounting for approximately 5.7% of all new cancer cases. Annually, around 1% of all deaths in the world were related to HCC [2]. However, with the advancement and development of screening diagnosis and treatment techniques, the early diagnosis rate, surgical resection rate, and postoperative survival rate of liver cancer patients have increased significantly. In addition, cancer-related symptoms and treatment measures impair both body function and the quality of life of patients [3]. As a result, cancer rehabilitation has been increasingly considered important for the treatment and prognosis of cancer patients. However, progress in relevant research progress has not been optimistic, and its achievements do not meet the demand. This is especially the case for cancer survivors after a long recovery period. Currently, there are still no authoritative and systematic postoperative rehabilitation guidelines [4,5]. Exercise therapy has potential therapeutic value for cancer patients regarding their quality of life, fatigue status, cardiopulmonary function, and side effects from radiotherapy and chemotherapy. Modern medicine has suggested that exercise therapy is one of the main treatments for postoperative rehabilitation of cancer patients. Exercise therapy can influence the recovery of cancer patients by changing hormone levels, inflammatory conditions, immune function, and other mechanisms. In addition, it can affect the body's material metabolism and energy metabolism. These reactions and adaptations will eventually be expressed in the form of metabolites. Metabolomics can track and detect the dynamic transformation and content changes of metabolites and link these metabolic information with biochemical and physiological changes in pathophysiological processes. Metabolomics can identify the target and action site of changes, and then determine the relevant biomarkers. The application of metabolomics to the field of exercise therapy can be used to detect the low molecular weight metabolites of exercise metabolism by spectroscopy technology, evaluate the effect of exercise intervention by using pattern recognition methodology, discover relevant biomarkers, and evaluate the metabolic regulatory network and its molecular mechanism of exercise [6].

The liver plays a major role in metabolism and has many functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, and detoxification. It is important to investigate the disorder of metabolisms in liver diseases. Therefore, it is important to systematically study the liver and liver diseases from the perspective of metabolism. While a large fraction of cancer metabolomic research is focused on finding diagnostic biomarkers for HCC, metabolomics is also being used to obtain more fundamental mechanistic insights into HCC. Applications of metabolomics are also emerging in areas such as tumor staging and assessment of treatment efficacy [7–9]. However, studies on metabolic changes of exercise therapy in HCC patients after surgery are limited, and research and clinical applications have not been given much attention. Therefore, in this study, mouse liver orthotopic xenograft tumor models were established, and treadmill exercise intervention was performed to evaluate the general state of mice. In addition, metabolite spectrums were obtained and used in the study of metabolomics to determine the duration and intensity of exercise interventions in mice.

Material and Methods

Experimental materials

A total of 60 healthy male C57Bl/6 mice, 6 to 8 weeks of age, weighing 20±2 g, were provided by Shanghai Sleek Laboratory Animal Co., Ltd. (SCXK, Shanghai, China, 2012-0002). Mice were housed in a clean environment, constant temperature (room temperature 22°C), constant humidity (relative humidity 50%), light from 06: 00 to 18: 00, free access to drinking water and food, cage specifications 25×15×18 cm. Each cage housed 5 animals. Aseptic surgery was performed in the experimental animal facilities of the Experimental Animal Center of the Second Military Medical University (Shanghai, China). All animal studies were approved by the Second Military Medical University Ethics Approval Committee under license number 13071002115.

Establishment and grouping of animals

Preparation of HCC transplantation tumor animal model was in brief: 5 mice were inoculated intraperitoneally with H22 cells (0.4 mL/mouse, 2×10^6 cells/mL). Mice with significant ascites were selected, and the ascites cells were collected and inoculated into the abdominal cavity of 5 new mice (0.4 mL/mouse, 2×10^6 cells/mL) to ensure high activity of the H22 cells. Next, mice with significant ascites were selected, and H22 cells in ascites were collected and resuspended in phosphate-buffered saline (PBS) to 2×10^6 cells/mL. Subsequently, 0.1 mL ascites was injected in the left forelimb of mice. When a grown to about 1 cm in diameter, the tumor tissue was cut into 2×2×2 mm pieces. Then I the experimental mice, the left lateral hepatic lobe was exposed, and a 3 mm deep tunnel was created with microsurgical forceps in the middle part of the left lateral lobe of the liver. The tumor tissue was implanted into the tunnel by using microsurgical forceps. The wound was slight pressed with a cotton swab for hemostasis and then sutured and closed. A small amount of penicillin was applied to prevent infection. After 10 days of implantation, entry into the abdomen along the original incision and suture of the left lobe of the liver with

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5-0 non-invasive needle thread at the back of the predetermined incision margin was performed, and hen resect of the left lateral lobe of the liver bearing the tumor. The incision margin was more than 0.5 cm away from the edge of the tumor. During and after the operation, the indoor temperature was maintained, and the mice were kept warm with blankets and sawdust, so as to avoid the death of mice caused by the inability to rewarm due to the low temperature [10].

We established a mouse treadmill model as treadmill running allows strict control of exercise intensity. This ensures the same relative intensity throughout the experimental period, which is essential for optimal outcomes. In treadmill protocols, both the duration and the intensity of exercise can be manipulated. So, the aerobic exercise intervention was adopted by using a mouse treadmill (Zhenghua Biological Instrument Equipment Co., Ltd., Huaibei, Anhui, China). The treadmill slope was set at 0°, the duration at 60 minutes, and the speed used included moderate-intensity: 12 m/minute or high intensity: 18 m/minute training once a day [11,12]. During the training process, the mouse was always stimulated with a brush to keep it in the front 1/3 of the runway. After the experiment, mice were evaluated for signs of injury. In case of any injury, they were treated accordingly. Mouse were trained 5 days a week for a total of 2 weeks, and a 10-minute warm-up period was allowed prior to each training (speed 1 m/minute, treadmill slope 0°). The sedentary control group was not subjected to treadmill intervention, and could move freely in the cage and were sacrificed at the same time point as the experimental group.

Groups

All mice underwent resection of orthotopic liver tumors on the same day and were divided into 6 groups (DAY1+12 m/min, DAY1+18 m/min, DAY1+Sedentary, DAY7+12 m/min, DAY7+18 m/min, and DAY7+Sedentary) with 10 mice per group. Exercise groups underwent a treadmill exercise plan involving 5 days per week for a total of 2 weeks. On the first postoperative day (early postoperative period) and the seventh postoperative day (late postoperative period), moderate-intensity or high-intensity treadmill exercise were started, and mice were sacrificed with the sedentary group after starting treadmill exercise.

Biochemical indicators

Mice were weighed after orthotopic excision and weighed again at the time of sacrifice. The 2 body weights were subtracted to obtain the body weight difference. Mice were monitored daily regarding mouse hair color, gait, and other general conditions. Ascites and death were recorded.

Blood was collected from the orbital vein at the time of sacrifice and serum were collected (4°C, 2500 rpm, 15 minutes) into BD Vacutainer® collection tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and stored at –80°C. Test for levels of alanine aminotransferase (ALT), aspartate transaminase (AST), albumin (ALB), globulin (Glob), and total protein (TP).

Metabolite sample collection and pretreatment

A total of 100 µL serum was added to 300 µL methanol (Merck & Co., Inc, Whitehouse Station, NJ, USA), the supernatant was collected after vortexed and centrifuged.

Serum metabolic profiling was performed using a Waters ACQUITY UPLC system (Waters Corp., Milford, MA, USA) coupled to Agilent 6538 UHD and Accurate-Mass Q-TOF/MS (Waters). Liquid chromatography conditions were as follows: mobile phase A was a 0.1% aqueous formic acid solution (Merck & Co., Inc.), mobile phase B was acetonitrile (0.1% formic acid), and a gradient elution was used to equilibrate the ACQUITY UPLC@ HSS T3 column (100Å, 1.8 µm, 2.1 mm; Waters). The column temperature was set to 40°C, the flow rate was 350 µL/minute, and a total of 4 µL was injected into the system.

Mass spectrometry conditions

Mass spectrometry uses an electrospray ion source and is detected using positive and negative ion modes. Positive ion mode detection parameters are as follows: capillary voltage 4000 V, drying gas flow rate 11 L/minute, drying gas temperature 350°C, spray pressure 45 psig, fragmentation voltage 120 V, Skimmer voltage 60 V, data acquisition range m/z 100 to 1100, selected internal standard ions of m/z 121.0509 and m/z 922.0098 were used to correct for real-time mass. Negative ion mode detection parameters were as follows: capillary voltage 3000 V, drying gas flow rate 11 L/minute, drying gas temperature 350°C, spray pressure 45 psig, fragmentation voltage 120 V, Skimmer voltage 60 V, data acquisition range m/z 100 to 1100, selected m/z 112.985587, and m/z 1033.988109 internal standard ions for real-time mass correction.

Metabolomics data analysis

To obtain a metabolic fingerprint profile, samples were analyzed by Waters UPLC/TOF.MS. The original mass spectral data was placed on the R software platform for peak extraction and screening. Subsequently, normalization and centering of the peaks were performed using the Metlab software platform, and a 3-dimensional visualization matrix including variables, observation and peak intensity was obtained. Simca-P software was used for multi-component statistical analysis of the data. Variables with variable importance (Variable Importance for the Projection, VIP) >1 were identified by UPLC analysis. At the same time, *t*-tests were used to evaluate differences between metabolites. Variables with a VIP of >1 and statistical

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Table 1. Effect of exercise intervention time and intensity on mortality and tumor recurrence rate.

Group	Mortality	Ascites formation rate
DAY1+12 m/min	1/10	0
DAY1+18 m/min	1/10	0
DAY1+SED	1/10	1/10
DAY7+12 m/min	1/10	2/10
DAY7+18 m/min	2/10	4/10
DAY7+SED	3/10	4/10

Table 2. Effects of exercise intervention time and intensity on body weight ($\bar{\chi}$±S, n=10).

Group	Weigh (g)
D1+12 m/min	–0.17±0.094
D1+18 m/min	1.68±0.29
D1+SED	1.11±0.50
D7+12 m/min	1.83±0.25
D7+18 m/min	2.85±0.88
D7+SED	1.82±0.83

Table 3. Effects of exercise intervention time and intensity on blood biochemical indicators ($\bar{\chi}$±S, n=10).

Group	AST (u/l)	ALT (u/l)	ALB (g/l)	Glob (g/l)	TP (g/l)	A/G (g/l)
D1+12 m/min	268.40±108.06	40.60±10.64	30.60±0.89	57.60±74.57	53.80±2.28	1.34±0.15
D1+18 m/min	377.60±136.11	39.20±2.77	28.80±3.96	25.80±1.30	54.60±5.02	1.12±0.13
D1+SED	238.25±28.43	62.75±37.12	30.25±1.26	23.75±2.50	54.00±2.94	1.27±0.13
D7+12 m/min	283.50±77.35	57.67±27.78	32.00±2.53*	25.83±1.17	57.83±2.71*	1.25±0.14
D7+18 m/min	364.50±263.93	66.00±31.87	32.12±2.53*	20.50±3.20	52.62±4.37*	1.59±0.2
D7+SED	318.77±175.93	48.78±14.7	26.44±4.10	23.67±3.04	48.77±4.41	1.12±0.25

* $P<0.05$; ** $P<0.01$, *vs.* sedentary group of the same period.

significance ($P<0.05$) were considered to have significant contributions to the model as potential biomarkers, and online databases HMDB (*http://www.hmdb.ca*), METLI (*http://www.metlin.scripps.edu*), KEGG (*http://www.genome.jp/keg/*) were used for comparison.

Data analysis

SPSS version 17.0 was used for statistical analyses. Data were expressed as the mean± standard deviation (X±S). Factorial analysis was performed using the F-test. Mean comparison was performed using multivariate analysis of variance. Differences between groups were compared using one-way analysis of variance (ANOVA), and $P<0.05$ was considered significant.

Results

Effects of different exercise intensities and exercise onset on liver function in mice

The mortality rate was higher in the post-operative exercise group when compared to the postoperative early exercise group. Moreover, the rate of formation of peritoneal effusion were higher in the postoperative group than the early exercise group (Table 1). After interaction analysis of the body weight of model mice, it cannot be concluded that there is an interaction effect between intervention time and intensity in the changes of mouse body weight, and the effect of time and intensity on mouse body weight changes are not significant (Table 2).

Factorial analysis showed that there was no interaction between time and intensity of exercise intervention on changes in AST, ALT, and GLOB levels, and no statistically significant effect of time and intensity was observed on liver function (Table 3).

Interaction analysis of ALB and TP levels showed that there was an interaction between intervention time and intensity (Table 3). The difference in the simple effects of exercise intensity at Day 7 postoperative intervention time level was statistically significant, as there were 3 levels of exercise intensity, the individual effects were compared in pairs. TP in the 12 m/minute group was 57.8±1.6 g/L, which was better when compared to that in the 18 m/minute group (TP: 52.6±1.4 g/L) and sedentary group (TP: 48.8±1.3 g/L). ALB of the 12 m/minute group (32.00±2.53 g/L), and the 18 m/minute group (32.12±2.53 g/L) were better when compared to that of the sedentary group (26.44±4.10 g/L), and the differences were

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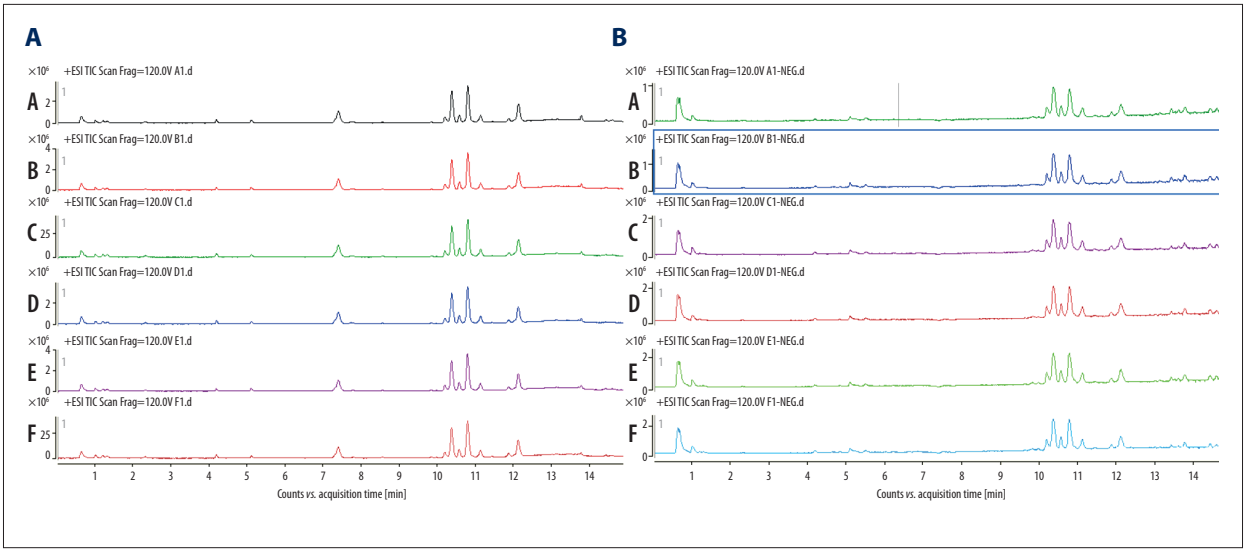

Figure 1. Total ion current (TIC), chromatograms. Positive (**A**) and negative (**B**) TIC chromatograms of representative serum samples from group A to F model mouse. Group A: early postoperative mice with 12 m/minute of treadmill exercise. Group B: early postoperative mice with 18 m/minute of treadmill exercise. Group C: early postoperative sedentary mice. Group D: late postoperative mice with 12 m/minute of treadmill exercise. Group E: late postoperative mice with 18 m/minute of treadmill exercise. Group F: late postoperative sedentary mice.

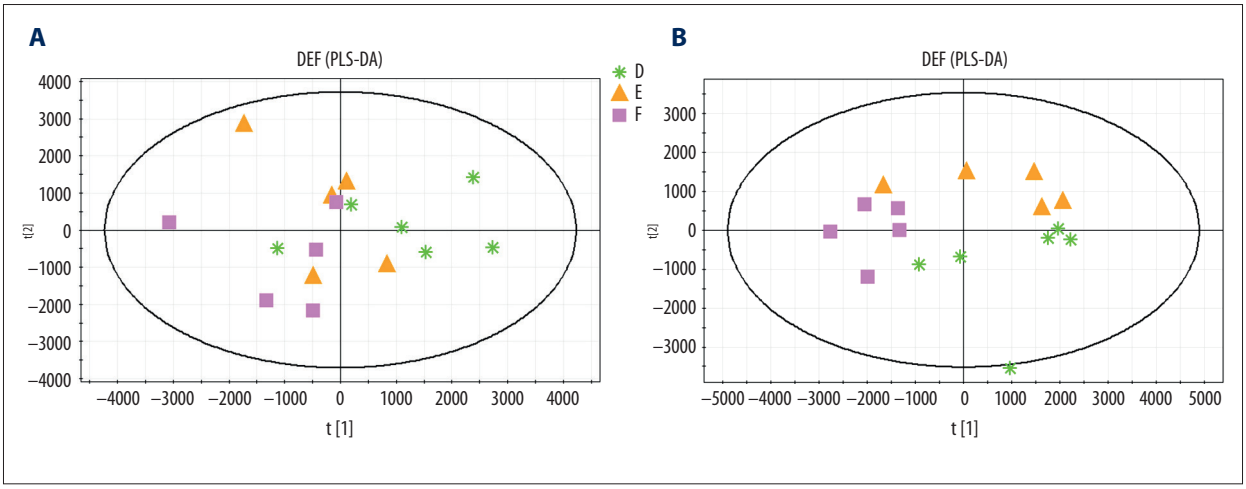

Figure 2. Partial least squares discrimination analysis (PLS-DA) score plot. Scores in positive (**A**) and negative (**B**) ion modes from the orthogonal partial least squares discrimination analysis (OPLS-DA) model classifying ultra performance liquid chromatography coupled to mass spectrometry (UPLC/MS) data from group D to F model mouse. Groups were named as described previously.

statistically significant ($P<0.05$). However, no significant differences were observed between the 12 m/minute group and the 18 m/minute group.

Multivariate analysis of mass spectrometry data

According to the previous chromatographic-mass spectrometry conditions, samples of each group were analyzed. The typical total ion chromatogram (TIC) in the positive and negative ion modes of samples in each group is shown in Figure 1.

Multivariate analysis was performed on the mass spectral data, and a supervised PLS-DA (partial least squares-discriminant analysis) approach was employed to obtain the Score plot of OPLS (orthogonal partial least squares), and Pareto scaling was used to perform OPLS-DA (orthogonal partial least squares-discriminant analysis). After performing PLS-DA, a clear separation of metabolic profiles was observed between groups on the negative ion pattern OPLS-DA scores, indicating metabolic differences between the three groups of samples (Figure 2).

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Table 4. Metabolic products of 12m/min treadmill exercise mice and sedentary mice on the first postoperative day.

Mode	Keep time Rt/min	Molecular ion (m/z)	Ion	Metabolites	Trend
ESI(+)	0.693717	203.053	M+Na/M+Na	D-Glucose	↑0.0459
	13.7292	282.28	M+H	Oleamide	↓0.03043
ESI(–)	191.02	1.0617	[M-H]-	Citric acid/Isocitrate	↓0.01320
	4.20141	203.083	M-H]-	L-Tryptophan	↓0.00253
	5.23292	212.002	[M-H]-	Indoxylsulfuric acid	↓0.0372
	11.1352	566.347	[M-H]-	PS (21: 0/0: 0)	↓0.0040
	10.399	612.331	[M+FA-H]-	PC (22: 6(4E,7E,10E,13E,16E,19E)/0: 0) [U]	↓0.0247
	12.1324	1091.73	[M-H]-	Ganglioside GA2 (d18: 1/18: 0)	↓0.01156

Table 5. Metabolic products of 18m/min treadmill exercise mice and sedentary mice on the first postoperative day.

Mode	Keep time Rt/min	Molecular ion (m/z)	Ion	Metabolites	Trend
ESI(+)	0.693717	203.053	M+Na	PC(20: 5(5Z,8Z,11Z,14Z,17Z)/18: 1(11Z))	↑0.0333
	13.7965	828.552	M+H	Oleamide	↓0.0338
ESI(–)	0.709471	124.008	[M-H]-	Taurine	↓0.0018
	12.1296	568.363	[M+FA-H]-	PAF C-16	↓0.0217

After comparing the 2 groups of PLS-DA, significant differences were observed between several metabolites, and the exact molecular weights of these differential metabolites were compared with those available in the following online databases: HMDB, METLI, and KEGG to determine the metabolic differences. As can be seen from Table 4, there are differences in various substances between the early 12 m/minute exercise group and the sedentary group under the positive and negative ion mode. D-glucose increased, L-tryptophan, oleamide, citric acid and taurine decreased. For the 18 m/minute exercise group at the same period, PC (phosphatidylcholine) (20: 5/18: 1) increased, PAF C-16, oleamide and taurine decreased shown as Table 5.

Compared with the sedentary group, the serum levels of LPE (lysophosphatidylethanolamine), indoxylsulfuric acid, PE (phosphatidylethanolamine) (16: 0/0: 0) and LPC (lysophosphatidylcholine) (16: 0) in the late 12 m/minute exercise group decreased, while pyruvate acid increased according to Table 6. The content of 5-hydroxytryptamine, tyramine acid, and succinic acid increased in serum of mice in late 18 m/minute exercise group increased. PE (18: 0/0: 0) and indolephenol decreased in both 12 m/minutes exercise group and 18 m/minute exercise group compared with the sedentary group according to Table 7.

Discussion

Evaluation of postoperative motor model of hepatocellular carcinoma (HCC) after surgery in mice

Mice have been used for establishing various disease models. However, literature to report the effects of treadmill exercise on mice after orthotopic liver tumor resection is limited. Therefore, in this study, we established and evaluated a mouse model of treadmill exercise after liver resection.

In this study, we found that 2 surgeries resulted insignificant damage to the liver of mice. Therefore, comprehensive indicators for the evaluation of hepatic function, including Glob, ALB, ALT, and AST were evaluated.

After factorial analysis, we found that ALB and TP levels in the treadmill exercise group were better when compared to those in the sedentary group in the late postoperative period. These differences were statistically significant and suggested that exercise inhibited the destruction of hepatocytes to some extent, improved glycogen metabolism, and promoted the recovery of liver function in mice. Moreover, this suggested that treadmill

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Table 6. Metabolic products of 12m/min treadmill exercise mice and sedentary mice on the seventh day post-surgery.

Mode	Keep time Rt/min	Molecular ion (m/z)	Ion	Metabolites	Trend
ESI(+)	12.07179	482.325	M+H/M+H/M+H	LysoPE(18: 0/0: 0)	↓0.02
ESI(–)	0.888634	133.014	[M-H]-/[M+FA-H]-	Pyruvate	↑0.04
	4.84816	178.051	[M-H]-/[M+FA-H]-	Indoxyl	↓0.015
	5.23292	212.002	[M-H]-	Indoxylsulfuric acid	↓0.001
	10.7393	452.279	[M-H]-	PE(16: 0/0: 0)	↓0.03
	12.0636	480.31	[M-H]-	PE(18: 0/0: 0)	↓0.002
	10.8034	530.302	M+Cl	LysoPC(16: 0)	↓0.0097

Table 7. Metabolic products of 18m/min treadmill exercise mice and sedentary mice on the seventh day after operation.

Mode	Keep time Rt/min	Molecular ion (m/z)	Ion	Metabolites	Trend
ESI(+)	2.03138	177.102	M+H	Serotonin	↑0.03
	2.03192	160.075	M+H/M+Na/M+Na/ M+Na/M+Na	Tyramine	↑0.02
ESI(–)	1.30073	117.02	[M-H]-	Succinic acid	↑0.016
	4.84816	178.051	[M-H]-/[M+FA-H]-	Indoxyl	↓0.012
	12.0636	480.31	[M-H]-	PE(18: 0/0: 0)	↓0.002

exercise in mice later in the postoperative period was more beneficial to the recovery of the general state of the mice.

Biochemical analysis and mechanism of action of identified metabolites

HCC has been the focus of metabolic analysis for many biomarker discovery and diagnostic models [7–9]. Studies have shown that metabolic abnormalities occur in HCC carcinogenesis, and approximately 105 metabolites show significant differences between cancer samples and healthy controls, such as glutamate, citric acid, taurine, ceramide, pyruvic acid, phenylalanine acid, oleic acid amide, lysophosphatidic acid, LPC, and so on [13,14]. These metabolites represented the abnormal metabolism, especially energy metabolic disorder, during the progress of hepatocarcinogenesis.

One of the key links of energy metabolism is tricarboxylic acid cycle. Obstacles of energy production and utilization in organisms may affect the metabolism of tricarboxylic acid cycle. Studies have shown that the tricarboxylic acid cycle in HCC cells was significantly higher than that in normal cells. In this study, treadmill exercise intervention was performed on model mice. Compared with sedentary model mice, levels of D-glucose increased, whereas levels of L-tryptophan, oleamide, and citric acid decreased in the early 12 m/minute exercise group. These findings indicated that the tricarboxylic acid cycle was affected by exercise, especially moderate exercise. It can lower the level of disorder tricarboxylic acid cycle. In addition, the serum tyramine acid and succinic acid expression levels increased, indicating that the late 18 m/minute exercise model mice had enhanced tricyclic carboxylase activity, mitochondrial dysfunction, and increased cancer related energy metabolism.

A truncated tricarboxylic acid cycle is one way of tumor energy metabolism and reorganization. In this study, moderate exercise significantly improved the disorder of the tricarboxylic acid in model mice, suggesting that exercise had an intervention effect on tumor-induced energy metabolism and reorganization. The main metabolic enzymes of tricarboxylic acid cycle exist in mitochondria of subcellular organelles, so the disorder of

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tricarboxylic acid cycle can indirectly reflect the dysfunction of mitochondria. Mitochondria play an important role in apoptosis and differentiation of cells. Therefore, the improvement of the disorder of metabolic intermediates of tricarboxylic acid cycle found drying gas temperature 350°C, spray pressure 45 psig, fragmentation voltage 120 V, may be a manifestation of an advance apoptotic disorder of cancer cells. In addition to the tricarboxylic acid related metabolites, serum taurine and serotonin levels increased, resulting in increased levels of internal reactive oxygen species (ROS), causing lipid peroxidation and other liver-related damage. Hepatotoxins are released through damaged hepatocytes, affecting bile acid formation and interfering with lipid metabolism. The proportion of oleic acid amide decreased in both the early moderate-intensity and the high-intensity exercise groups and was lower when compared to that in the sedentary group, suggesting that exercise has a protective effect on the destruction of lipid metabolism.

In the postoperative exercise intervention group, metabolites of PS (phosphatidylserine), PC, PE, LPE, pyruvate, PE (16: 0/0: 0), and PE (18: 0/0: 0) also changed. PS, PC, and PE are components of cell membrane phospholipids, and their abnormal distribution may result in apoptosis. LPC is hydrolyzed by phosphatidylcholine through phospholipase A2, which is involved in cell proliferation, tumor cell invasion, and inflammation. Liver is the main site of LPC biosynthesis, and its metabolic disorders are thought to lead to liver injury and tumorigenesis and development [15]. LPE 16: 0, and LPC 22: 5 were defined as "marker metabolites", which can be used to distinguish the different stages of hepatocarcinogenesis and represent the abnormal metabolism during the progress of HCC in patients [16]. The escape of cell membrane PS has become an important indicator of cell apoptosis and together with PE is involved in the formation of apoptotic lipid rafts [17]. LPE has a strong surface activity and can further aggravate the destruction of cell membranes. A variety of tumors and treatment-related stress states have been reported to cause abnormal activation of phospholipase A2 [18,19]. Moreover, the destruction of membrane phospholipids is exacerbated, and PE is released from the cell membrane into the blood, thereby further destroying the membrane, including the organelle membrane [20]. On the one hand, this process destroys the integrity of the mitochondrial membrane, leading to changes in permeability in the mitochondrial permeability transition pore. This causes the outer membrane to rupture, releasing pro-apoptotic factors, and promoting apoptosis through caspase-dependent and/or non-caspase-dependent pathway mechanisms. On the other hand, lipid peroxides can stimulate the release of calcium ions stored in mitochondria, the endoplasmic reticulum, or sarcoplasmic reticulum, and induce apoptosis in cells [21]. In this study, the PE content in the treadmill exercise intervention group decreased when compared with that in the control group. This may be because exercise improves the stress state caused by liver cancer and surgical treatment, and reduces abnormal phospholipase activity, thereby reducing cell membrane destruction, and decreasing PE and LPE, thereby inhibiting apoptosis induced by the mitochondrial pathway. These findings reflected that aerobic exercise improved the stress response after liver cancer in mice.

The increase in metabolites in liver cancer patients reflects an increase in energy metabolism in the body, and a disordered tricarboxylic acid cycle. Fukuda et al. reported a significant increase in the tricarboxylic acid cycle in hepatoma cells when compared to normal cells. Because the main metabolic enzyme of the tricarboxylic acid cycle exists in the subcellular organelle mitochondria, a disordered tricarboxylic acid cycle can indirectly reflect in dysregulation of mitochondrial function, thereby affecting cell apoptosis and differentiation. Therefore, the disordered metabolic intermediates found in the tricarboxylic acid cycle, discovered through metabolomics studies, is a manifestation of disordered apoptosis in tumor cells. In this study, the major metabolic products involved energy metabolites and phospholipid metabolites, suggesting that exercise improved the tricarboxylic acid cycle and mitochondrial dysfunction, reduced energy metabolism, and inhibited mitochondrial pathway-induced apoptosis.

Conclusions

Studies performed after treadmill exercise with different intervention times and intensities of liver transplanted orthotopically transplanted tumors revealed that there was no statistically significant effect of exercise intervention on changes in body weight. Moreover, liver function was better when compared to that of mice in the sedentary group. Through multivariate statistical analysis and comparison of databases, we showed that the metabolic products in the early postoperative exercise group mainly involved energy metabolites, such as D-glucose and L-tryptophan. However, in the post-operative period, serum serotonin, tyramine, and succinic acid levels increased in the high-intensive exercise group, except for energy metabolites. In the moderate-intensity group PE (18: 0/0: 0) and taurine levels decreased and the phospholipid metabolite levels such as LPE, pyruvate, PE (16: 0/0: 0), and LPC (16: 0) were decreased.

These findings suggested that aerobic exercise reduced the degree of postoperative stress response, promoted the restoration of energy metabolism balance, inhibited apoptosis, and improved liver function. The effect of moderate-intensity exercise intervention was most significant in the late postoperative period. The mechanism involved improving the tricarboxylic acid cycle, intervening with energy metabolism and reorganization caused by tumors, reducing the abnormal increase of

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phospholipase activity caused by postoperative hepatocellular carcinoma stress, decreasing the level of hemolytic phospholipids, and thereby inhibiting the mitochondrial pathway apoptosis. Such findings may have important implications for clinically reducing postoperative stress response and promoting rapid recovery after liver cancer surgery.

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Conflicts of interest

None.

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