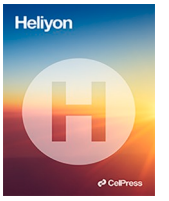
Heliyon 10 (2024) e25562

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Low-intensity exercise training improves systolic function of heart during metastatic melanoma-induced cachexia in mice

Lin Wang [a,1], Xuchao Wang [a,1], Jingyu Chen [a], Yang Liu [a,b], Gang Wang [a], Linjian Chen [a], Wei Ni [a], Yijia Jia [c], Cuilian Dai [a], Wei Shao [a,**], Binbin Liu [a,*]

[a] Xiamen Cardiovascular Hospital, School of Medicine, Xiamen University, Jinshan Road 2999, Xiamen, 361015, China
[b] Liaoning University of Traditional Chinese Medicine, Chongshan East Road 79, Shenyang 110032, China
[c] Zhoukou Central Hospital, Renmin Road 26, Zhoukou, 466000, China

ARTICLE INFO

Keywords:
Systolic function
Heart
Exercise
Cancer cachexia
Melanoma

ABSTRACT

Cardiac dysfunction frequently emerges in the initial stages of cancer cachexia, posing a significant complication of the disease. Physical fitness is commonly recommended in these early stages of cancer cachexia due to its beneficial impacts on various aspects of the condition, including cardiac dysfunction. However, the direct functional impacts of exercise on the heart during cancer cachexia largely remain unexplored. In this study, we induced cancer cachexia in mice using a metastatic B16F10 melanoma model. Concurrently, these mice underwent a low-intensity exercise regimen to investigate its potential role in cardiac function during cachexia. Our findings indicate that exercise training can help prevent metastatic melanoma-induced muscle loss without significant alterations to body and fat weight. Moreover, exercise improved the melanoma-induced decline in left ventricular ejection fraction and fractional shortening, while also mitigating the increase in high-sensitive cardiac troponin T levels caused by metastatic melanoma in mice. Transcriptome analysis revealed that exercise significantly reversed the transcriptional alterations in the heart induced by melanoma, which were primarily enriched in pathways related to heart contraction. These results suggest that exercise can improve systolic heart function and directly influence the transcriptome of the heart during metastatic melanoma-induced cachexia.

1. Introduction

Cachexia is a debilitating syndrome marked by the gradual depletion of muscle mass, significant weight loss, and reduced cardiac function in advanced cancer patients, including those with metastatic melanoma [1]. In cancer patients, an unintentional loss of >5 % body weight is defined as cachexia, and it presents as a major complication of the disease [2]. Frequently observed in the terminal phases of numerous chronic diseases, cachexia affects around 50 %–80 % of patients with solid tumors and accounts for one-third of all cancer-related deaths [3,4]. The incidence and severity of cachexia increase with metastatic progression, suggesting a potential molecular link between cachexia development and the metastatic process [5,6]. Despite several studies suggesting that preventing

* Corresponding author. Xiamen Cardiovascular Hospital, Xiamen University, Jinshan Road 2999, Xiamen, 361015, China.
** Corresponding author. Xiamen Cardiovascular Hospital, Xiamen University, Jinshan Road 2999, Xiamen, 361015, China.
E-mail addresses: vivishao@foxmail.com (W. Shao), liubinbin@xmu.edu.cn (B. Liu).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e25562
Received 6 August 2023; Received in revised form 23 January 2024; Accepted 29 January 2024
Available online 6 February 2024

muscle mass loss in tumor-bearing mice can improve prognosis and extend survival, the underlying molecular mechanisms remain elusive. As of now, there are no drugs approved by the FDA specifically for the treatment of cachexia [7,8].

Moreover, cachexia exhibits varied impacts on different tissues, with the heart and skeletal muscle particularly affected in the early stages [9]. Patients with cancer cachexia often manifest impaired cardiac function [10]. For instance, stage IV non-small cell lung cancer patients experienced a progressive decrease in the systolic function of the heart. Systolic function measures, including the left ventricular ejection fraction (LVEF) and global longitudinal strain (GLS), determined by echocardiography, exhibited a significant decline at 4 months compared to baseline [11]. While impaired mitochondrial function, increased oxidative stress, and autophagy are known contributors to the cardiac dysfunction observed during cancer-induced cachexia, the detailed mechanisms underlying these processes remain largely unknown [10,12].

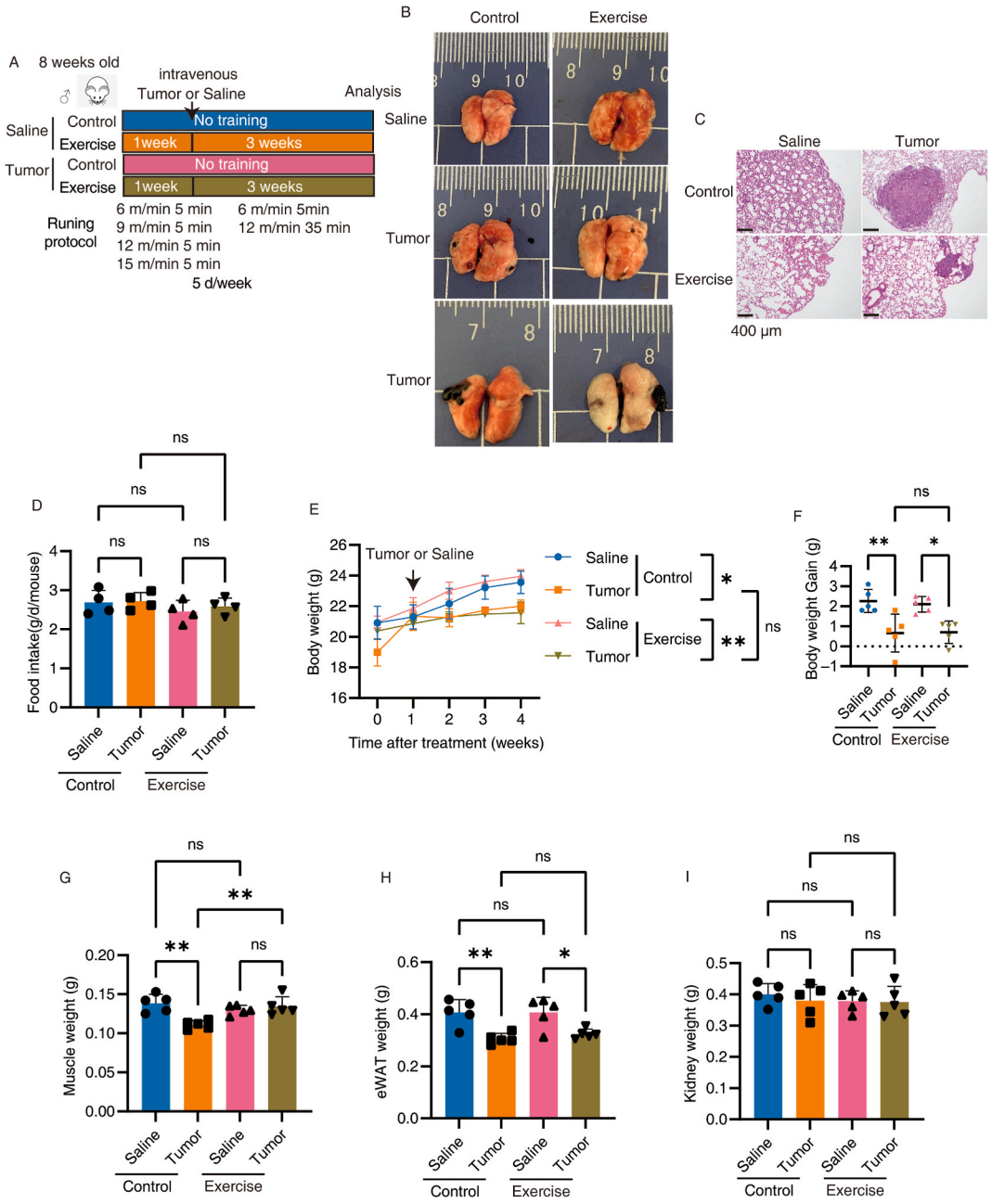


Fig. 1. Exercise training mitigates skeletal muscle loss during cancer cachexia. (A) Outline of the experimental procedure and training protocol. **(B)** Representative images illustrating the lung and metastatic tumors. **(C)** Representative images of H&E staining of lung tissue. **(D)** Food intake in mice across different groups. **(E)** Body weights of mice. **(F)** Body weight change in specified groups 3 weeks post-tumor injection. Weights of muscle **(G)**, epididymal white adipose tissue (eWAT) **(H),** and kidney **(I)**. Data are presented as mean ± SD. n = 5.

Physical fitness maintenance is widely recommended in the early stages of cancer cachexia [13,14], and the American College of Sports Medicine (ACSM) has formulated exercise guidelines for cancer patients due to its critical role in enhancing both the quantity and quality of life for cancer survivors [15,16]. Exercise has been reported to counteract the most prominent mechanisms and symptoms of cancer cachexia, such as muscle wasting, reduced appetite, anorexia, increased energy expenditure, fat wasting, and cardiac dysfunction [17]. This underscores the potential of exercise as a valuable intervention in managing various aspects of cachexia. One of the major underlying mechanisms behind the benefits of exercise is its ability to induce a systemic anti-inflammatory response and enhance mitochondrial fitness [18,19]. Furthermore, physical activity is widely acknowledged for its potential to reduce the risk of cardiovascular disease through diverse mechanisms. These mechanisms involve altering metabolism via elevating circulating levels of high-density lipoprotein, enhancing insulin sensitivity, and modulating the balance between anti- and pro-inflammatory responses. This modulation is achieved by promoting the secretion of anti-inflammatory cytokines, elevating the counts of circulating IL-10 producers, and reducing the levels of Toll-like receptors on monocytes [20,21]. Despite these known benefits of exercise, the specific effects of exercise on the cardiac function and cardiomyocytes during cancer cachexia remain largely unknown. Thus, we aimed to understand the direct effects of exercise on the heart during cancer cachexia in mice.

Here, we utilized a metastatic melanoma model to induce cancer cachexia in mice [9,22]. Concurrently, we subjected the mice to a low-intensity exercise regimen to investigate the potential role of exercise in cardiac function during cachexia. We employed echocardiography and transcriptome analysis to assess the impact of exercise on cardiac function in the context of cancer cachexia. Our findings revealed that exercise training led to improvements in the cardiac systolic function and caused significant alterations in genes associated with heart contraction during cancer cachexia. These findings imply that physical exercise may be advantageous for individuals undergoing cardiac dysfunction in the context of cancer cachexia.

2. Materials and methods

2.1. Cell culture

Mouse melanoma B16F10 cells (CL-0319, Procell, Wuhan, China) were cultured in RPMI 1640 medium (L210KJ, basalmedia, Shanghai, China), supplemented with 10 % fetal bovine serum (AC03L055, Life-iLab, Shanghai, China) and 1 % penicillin-streptomycin solution (PB180120, Procell). The cells were maintained at 37 °C in a humidified atmosphere with 5 % CO_2.

2.2. Animal study

Male C57BL/6J mice, aged 8 weeks were kept in specific pathogen-free facilities and categorized into a low-intensity exercise group and a control group based on our previous publication [23]. Mice in the exercise group engaged in a treadmill running regimen for 4 weeks, with 2 days off each week. During the initial week, mice underwent adaptive training as follows: 6 m/min for 5 min, 9 m/min for 5 min, 12 m/min for 5 min, and 15 m/min for 5 min. Then they ran at 6 m/min for 5 min and 12 m/min for 35 min for additional 3 weeks. In parallel, control mice were housed in cages without engaging in exercise. Starting from the second week of the study, 1×10^5 B16F10 cells were intravenously injected into the mice through the tail vein to induce cancer cachexia [9,22]. (Fig. 1A).

Body weight and food intake of the mice were measured every week throughout the duration of the experiments. At day 21 post-tumor injection or upon reaching human endpoints, the mice were euthanized using CO_2 inhalation in accordance with ethical guidelines.

2.3. Echocardiography

Transthoracic echocardiography for evaluating cardiac systolic function was performed on day 20 of the study using a Vevo 2100 linear imaging system (FUJIFILM, Tokyo, Japan) with anesthesia by inhaling 1.0–2.25 % isoflurane (RWD, China). Image acquisition occurred with the heart rate maintained between 450 and 550 beats per minute, utilizing the 22–55 MHz linear array transducer (MS-550D, FUJIFILM) under consistent conditions. Echocardiographic data were analyzed using Vevo Lab software (v3.2.6). Throughout the process of data acquisition and analysis, the investigators remained unaware of the treatment status of individual mice.

2.4. RNA-seq and Bioinformatic analysis

The RNA was extracted from the whole heart of the mice using Trizol reagent (15596026, Thermo Fisher Scientific, Waltham, U.S.) following the manufacturer's instructions. A cDNA library was subsequently prepared using the VAHTS Universal V8 RNA-seq Library Prep Kit for MGI (NRM605-01, Vazyme, Nanjing, China). The resulting cDNA library was pooled and sequenced using DNBSEQ-T7 (MGI, Shenzhen, China), generating 150-bp paired-end reads. The obtained sequencing reads were aligned to the reference mouse genome GRCm39 using HISAT 2.2.1. Read counts per gene locus were calculated using featureCounts. The statistical analysis was executed using R, and the clustering of gene expression trends was conducted using the TCseq package (version 3.17, https://doi.org/10.18129/B9.bioc.TCseq), utilizing the mean expression of each gene [24,25]. The clustering algorithms contains hierarchical, pam, kmeans and fuzzy cmeans. The identification of significantly differentially expressed genes (DEGs) involved the utilization of the edgeR package, where a fold change (FC) of $\geq$1.5 and a p-value $<$0.05 were considered as criteria for significance. Furthermore, pathway enrichment analysis was conducted using the clusterProfiler package.

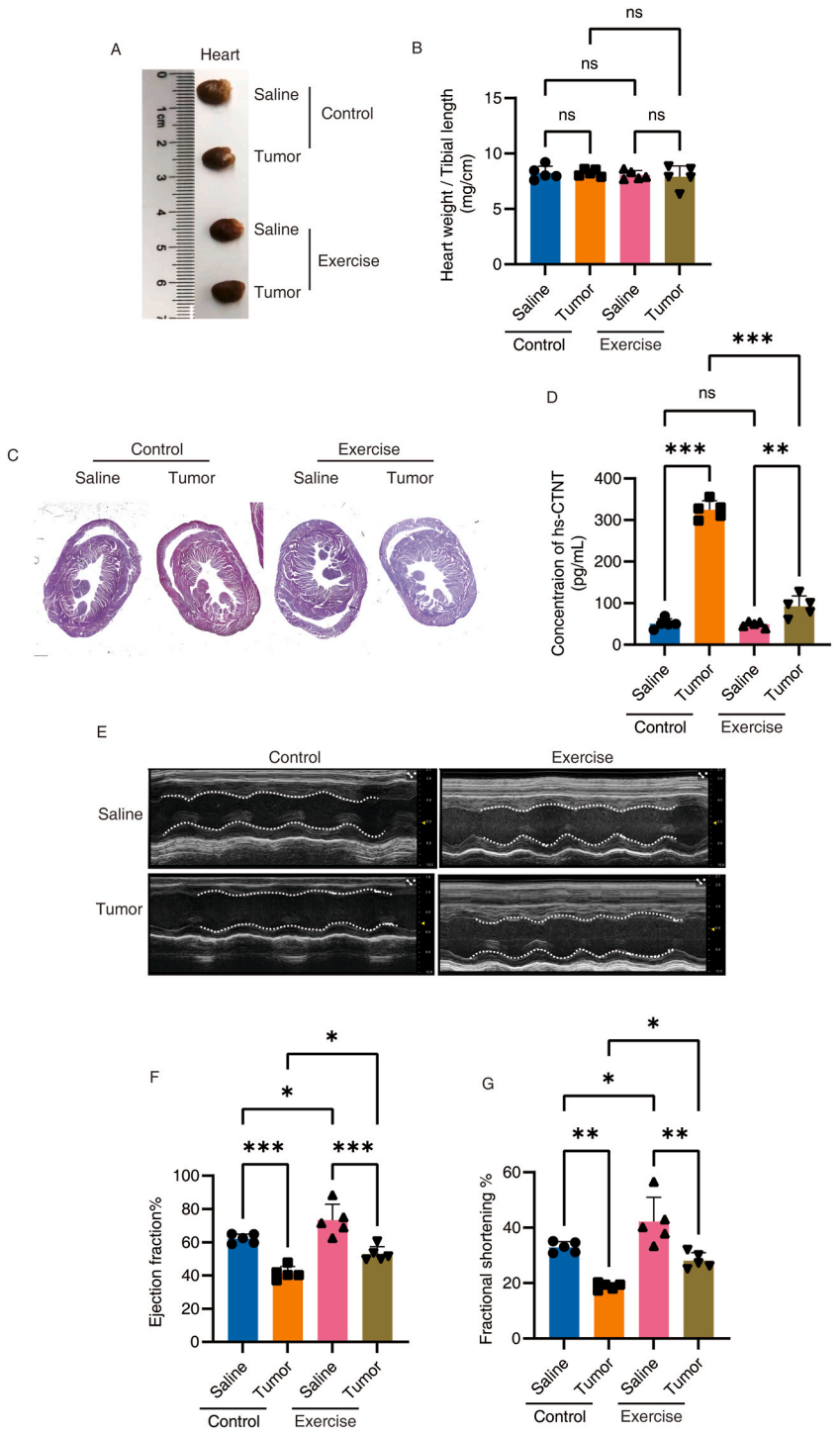


Fig. 2. Exercise training enhances systolic function of the heart during cancer cachexia. (**A**) Representative images of the heart. (**B**) Ratio of heart weight to tibia length in mice. (**C**) Representative H&E staining images of the heart. (**D**) Concentration of high-sensitive cardiac troponin T (hs-cTnT) in serum as measured by ELISA. (**E**) Representative echocardiographic M-mode images of the indicated mice in the parasternal short-axis view. (**F**) Ejection fraction (EF%) of the indicated mice. (**G**) Fractional shortening of the indicated mice. Data are presented as mean ± SD. n = 5.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Serum, obtained from clotted blood samples, was used to measure the concentrations of high-sensitive cardiac troponin T (hs-cTnT). The measurements were performed using ELISA kits (KT99892, MSKbio, Wuhan, China) following the manufacturer's instructions.

2.6. Hematoxylin and Eosin (H&E) staining

Lung and heart tissues were fixed in 4 % paraformaldehyde, after which paraffin sections were prepared. Hematoxylin and Eosin (H&E) staining was performed using an H&E staining kit (C0105 M, Beyotime, Shanghai, China) following the manufacturer's instructions.

2.7. Western blot analysis

Equivalent amounts of total protein, extracted from heart tissue using RIPA buffer (P0013B, Beyotime), were subjected to electrophoresis on SDS-PAGE. The standard Western blotting procedures involved the transfer of proteins to a PVDF membrane [26]. Following blocking with 3 % skim milk in TBST buffer, primary antibodies against MYH7B (#A19246, Abclonal, Wuhan, China), and β-actin (#AC026, Abclonal) diluted in 3 % BSA-TBST buffer were incubated at 4 °C overnight. After washing, membranes were exposed to the appropriate HRP-conjugated secondary antibodies, and protein bands were visualized using ECL reagent. The complete gel images are available in the Supplementary Material.

2.8. Statistics

Prism 9 software was utilized for statistical analysis, and the data were expressed as mean ± standard deviation (SD). ANOVA with Tukey's multiple comparisons test was applied after assessing normality using Shapiro-Wilk test and comparing the variances by Brown-Forsythe test. Two-way ANOVA with Šídák's multiple comparisons test was applied in Fig. 1E. Statistical significance was considered at p values ≤ 0.05. The figures represent p values as follows: not significant (NS), $p > 0.05$; *, $p \leq 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

3. Results

3.1. Exercise training ameliorates the loss of skeletal muscle during cancer cachexia

To investigate the role of exercise in cardiac cachexia, we intravenously injected B16F10 melanoma cells into mice and subjected them to treadmill running exercise (Fig. 1A). At 21 days after tumor injection, we observed metastatic tumors induced by B16 cells in the lungs of the tumor-bearing mice. However, exercise did not significantly reduce the size of these tumors (Fig. 1B). H&E staining also suggested that the histological structures of both the tumor and lung did not show clear differences after exercise in tumor-bearing mice (Fig. 1C).

Throughout the exercise period, we monitored the food intakes, which remained consistent among different groups (Fig. 1D). The body weight decreased by 6.62 %, and the body weight gain was reduced from 2.26 ± 0.57 to 0.66 ± 0.95, a 70.8 % reduction, after tumor injection in mice, indicating that cancer cachexia occurred, as unintentional loss of >5 % body weight is defined as cachexia. However, the body weight loss was observed regardless of whether the mice were subjected to exercise training (Fig. 1E and F). Additionally, the skeletal muscle weight significantly decreased after tumor injection in mice without exercise, but not in mice with exercise (Fig. 1G). The weights of the epididymal white adipose tissue (Fig. 1H) and kidney (Fig. 1I) did not show any significant differences among all the groups. These results suggested that the B16F10 tumor could induce cancer cachexia in mice, and exercise has the potential to prevent the loss of skeletal muscle and ameliorate cancer cachexia.

3.2. Exercise training elevates cardiac systolic function during cancer cachexia

To understand the impact of exercise training on cancer-induced cardiac cachexia, we analyzed cardiac functions. Firstly, we assessed the size of the heart and the ratio of heart weight to tibial length after tumor injection and exercise training. However, we did not observe significant changes in these parameters in response to either tumor injection or exercise training (Fig. 2A and B). Histological examination using H&E staining also revealed that neither tumor presence nor exercise training caused significant alterations in the histological structure of the heart (Fig. 2C). These observations suggest that neither the tumor nor the exercise had a notable effect on the overall size or the cardiac tissue of the heart.

Next, we evaluated cardiac function by measuring the concentration of high-sensitive cardiac troponin T (hs-cTnT), an indicator of myocardial injury [27,28]. Interestingly, we found that the presence of a tumor led to an increase in hs-cTnT concentration, whereas exercise significantly reversed the tumor-induced increase in hs-cTnT concentration (Fig. 2D). This suggests that the increased cardiac injury caused by the tumor could be mitigated by exercise. To further assess the systolic function of the heart, we used an echocardiogram to examine the structure and function of the heart. Our results demonstrated that the tumor caused decreases in both EF% and fractional shortening (FS%) of LV, indicating impaired systolic function of the heart in response to the tumor (Fig. 2E–G). Moreover,

Wang L. *et. al.*

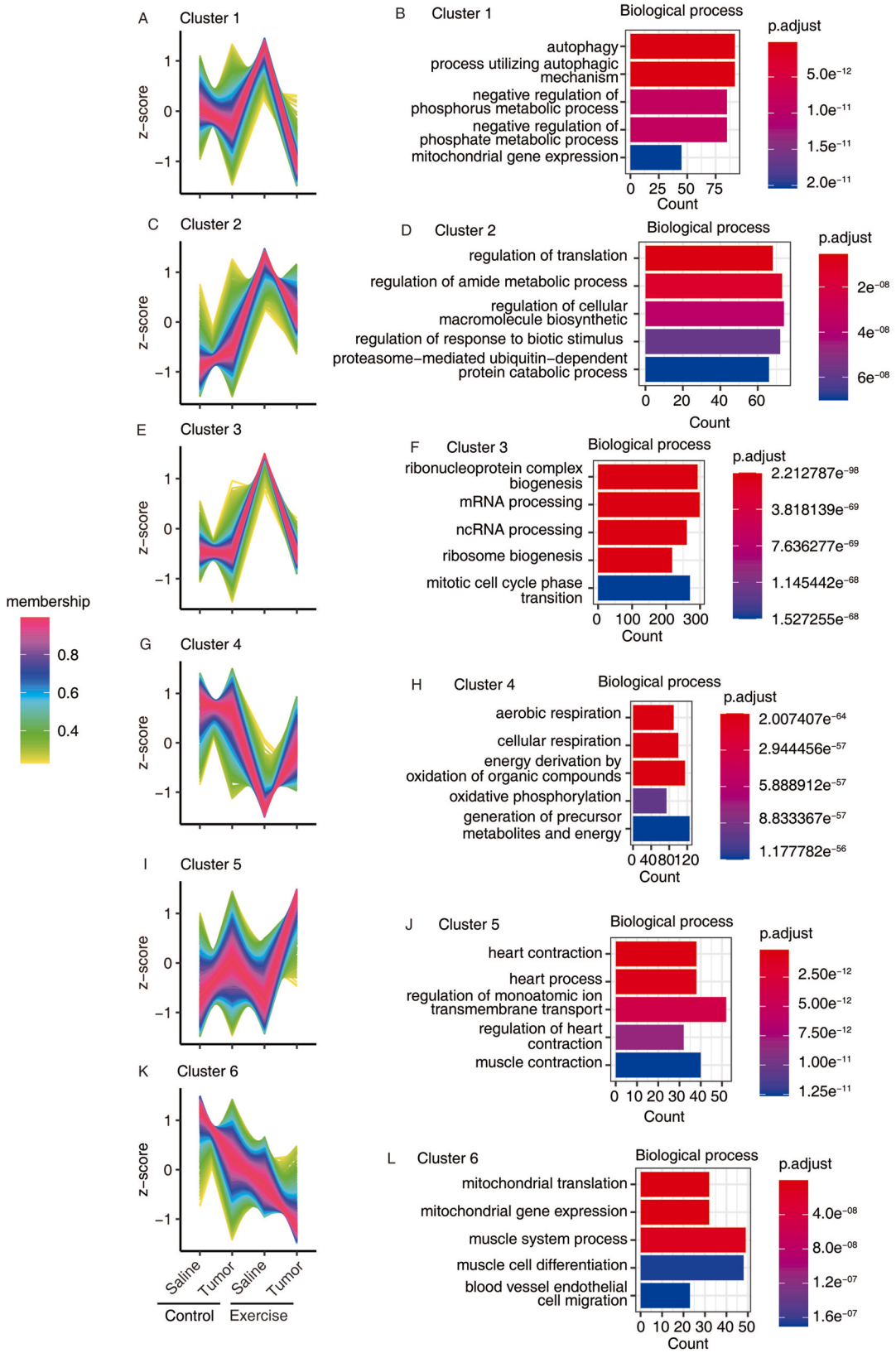

A
Cluster 1
B
Cluster 1
Biological process
p.adjust
autophagy
process utilizing autophagic mechanism
negative regulation of phosphorus metabolic process
negative regulation of phosphate metabolic process
mitochondrial gene expression
0 25 50 75
Count
5.0e−12
1.0e−11
1.5e−11
2.0e−11
C
Cluster 2
D
Cluster 2
Biological process
p.adjust
regulation of translation
regulation of amide metabolic process
regulation of cellular macromolecule biosynthetic
regulation of response to biotic stimulus
proteasome−mediated ubiquitin−dependent protein catabolic process
0 20 40 60
Count
2e−08
4e−08
6e−08
E
Cluster 3
F
Cluster 3
Biological process
p.adjust
ribonucleoprotein complex biogenesis
mRNA processing
ncRNA processing
ribosome biogenesis
mitotic cell cycle phase transition
0 100 200 300
Count
2.212787e−98
3.818139e−69
7.636277e−69
1.145442e−68
1.527255e−68
membership
0.8
0.6
0.4
G
Cluster 4
H
Cluster 4
Biological process
p.adjust
aerobic respiration
cellular respiration
energy derivation by oxidation of organic compounds
oxidative phosphorylation
generation of precursor metabolites and energy
0 40 80 120
Count
2.007407e−64
2.944456e−57
5.888912e−57
8.833367e−57
1.177782e−56
I
Cluster 5
J
Cluster 5
Biological process
p.adjust
heart contraction
heart process
regulation of monoatomic ion transmembrane transport
regulation of heart contraction
muscle contraction
0 10 20 30 40 50
Count
2.50e−12
5.00e−12
7.50e−12
1.00e−11
1.25e−11
K
Cluster 6
L
Cluster 6
Biological process
p.adjust
mitochondrial translation
mitochondrial gene expression
muscle system process
muscle cell differentiation
blood vessel endothelial cell migration
0 10 20 30 40 50
Count
4.0e−08
8.0e−08
1.2e−07
1.6e−07
z−score
1
0
−1
Saline
Tumor
Saline
Tumor
Control
Exercise

Fig. 3. Exercise training potentially reverses tumor-induced alterations in the heart's transcriptional profile. RNA-seq was conducted using heart samples, with each sample comprising tissue from two mice (n = 3). Gene clustering analysis was conducted based on the mean expression of each gene using the TCseq package (**A, C, E, G, I, and K**) Trends of gene expression among each group. (**B, D, F, H, J, and L**) Gene Ontology term enrichment analysis of indicated gene cluster. (**M and N**) The principal component analysis (PCA) plots of the indicated groups are displayed. The dot represents the centroid of the PCA scores of each group, and the curve indicates the standard deviation range of the PCA scores.

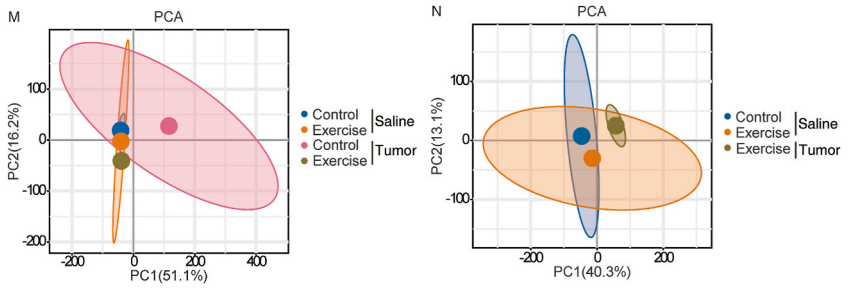


Fig. 3. (*continued*).

exercise training positively affected cardiac functions, as evidenced by increased EF% and FS% of LV in both tumor-free and tumor-bearing mice (Fig. 2E–G). Overall, our data suggest that exercise training could improve the systolic function of the heart during cancer-induced cardiac cachexia, offering potential benefits for mitigating cardiac dysfunction associated with cachexia.

Exercise training exercise has the potential to reverse the tumor-induced alterations in the transcriptional profile of the heart.

To gain deeper insight into the impact of exercise on cardiac function during cachexia, we performed RNA sequencing analysis and categorized genes into six clusters based on their expression patterns. We observed that exercise training repressed the tumor-induced upregulation of certain genes in clusters 1, 2, and 3. Gene Ontology (GO) enrichment analysis indicated that these genes were associated with autophagy, translation, and RNA processing, respectively (Fig. 3 A-F). Additionally, we found that exercise training increased the expression of genes in clusters 4 and 5, which were downregulated by the tumor. The functions of these genes were linked to aerobic respiration, energy production, heart contraction, and muscle contraction (Fig. 3G–J). Moreover, we observed a decrease in genes associated with mitochondrial functions in cluster 6 after tumor injection, which was further reduced after exercise (Fig. 3K-L).

To comprehend the comprehensive transcriptional alterations induced by tumor and exercise, we conducted a principal component analysis (PCA). The distances among the control, exercise in tumor-free mice, and exercise in tumor-bearing mice are relatively smaller than the distance to the tumor-only group. Additionally, PCA was employed to elucidate the transcriptome variances among the control and exercise in tumor-free and tumor-bearing mice. An evident distinction still exists among them, suggesting that the transcriptome undergoes significant changes following tumor or exercise addition. Notably, exercise appears to counteract this alteration in tumor-bearing mice (Fig. 3M and N).

Overall, the results revealed that exercise could potentially reverse the tumor-induced alterations in the transcriptional profile of the heart, particularly related to autophagy, heart contraction, and mitochondrial functions.

3.3. Exercise training significantly elevated the genes associated with heart contraction during cancer-induced cardiac cachexia

Next, we investigated the significantly differentially expressed genes (DEGs) in the heart induced by both tumor and exercise. First, we observed that the tumor significantly upregulated 741 genes, of which 318 were significantly repressed by exercise (Fig. 4A). This finding suggests that exercise has the ability to counteract the tumor-induced upregulation of these genes. Gene ontology (GO) enrichment analysis showed that the functions of these genes were associated with the regulation of heart contraction and blood circulation (Fig. 4B). Included in this group were genes such as *Myh7b*, encoding a typical sarcomeric myosin protein [29], and *Pde4d* [30], *Rnf207* [31], *Tmem65* [32] and *Kcnj5* [33] which encode ion channel-associated proteins (Fig. 4C). Western blot analysis confirmed that exercise training reverse tumor-induced decreased protein levels of MYH7B in the hearts of tumor-bearing mice (Fig. 4D), indicating that exercise may improve cardiac myosin loss and to counteract tumor-induced cardiac dysfunction. Furthermore, we identified 2020 genes that were significantly increased after tumor injection, and among them, 1447 genes were significantly repressed by exercise (Fig. 4E). These genes were associated with cell cycle and DNA replication processes (Fig. 4F). Overall, our findings suggest that exercise training can significantly counteract tumor-induced transcriptional changes in the heart, particularly those related to the enhancement of heart contraction.

4. Discussion

Progressive loss of body weight along with decreased muscle was observed in our mouse model of cancer cachexia using B16F10 melanoma cells. A comparable decrease in body weight over time was reported in another cachectic melanoma model utilizing orthotopic pediatric melanoma xenografts [9], indicating that our tumor model could effectively induce cachexia. However, we observed relatively weaker metastasis compared to findings published in other studies using similar amounts of B16F10 cells [34,35]. Such variations might result from differences in cell culture conditions, mouse strains, or environmental factors. One difference in our

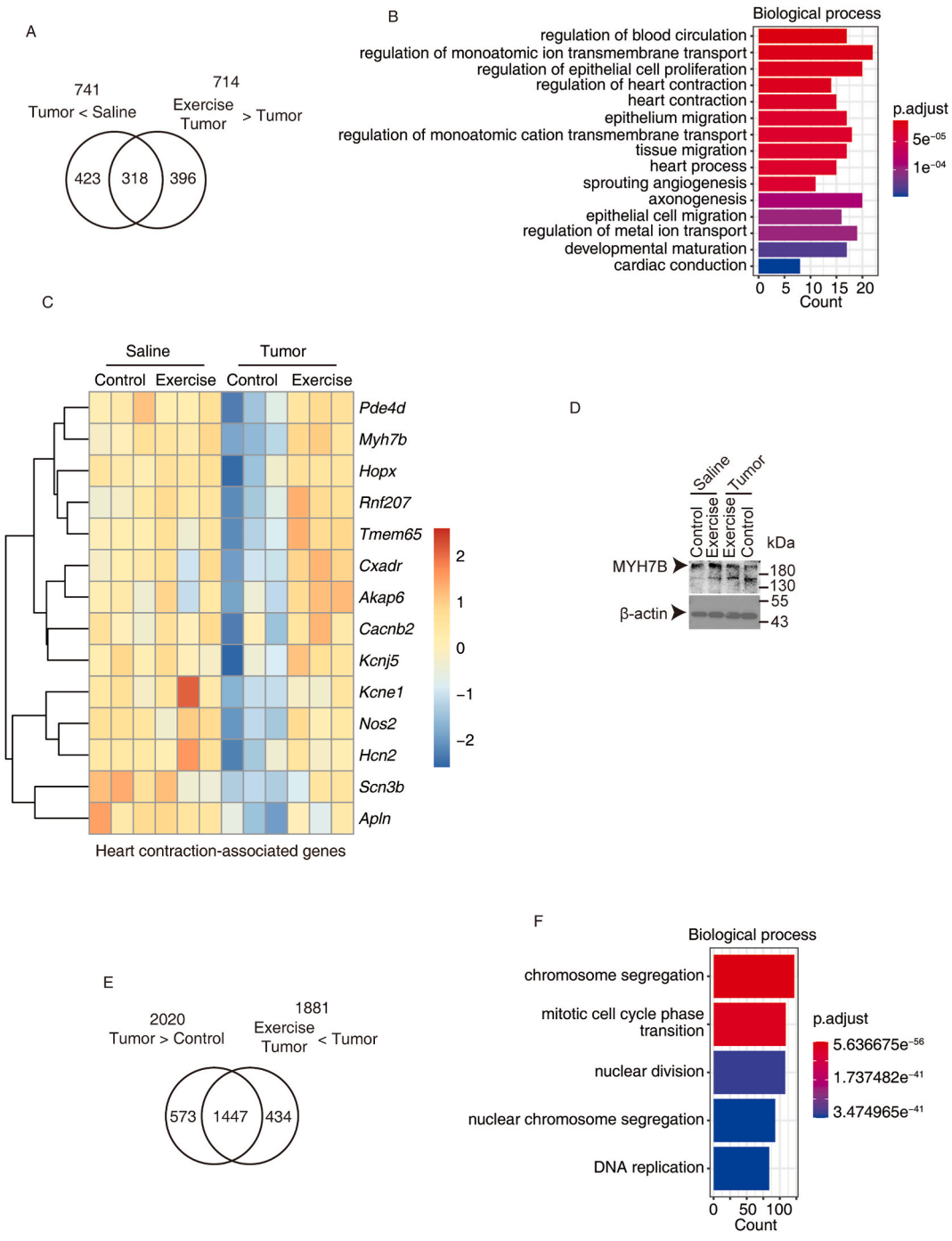


Fig. 4. Exercise training significantly elevated the genes associated with heart contraction during cardiac cachexia. (**A**) Venn diagram displaying the overlapping genes. (**B**) Gene Ontology term enrichment analysis of the overlapping genes identified from A. (**C**) Heatmap of heart contraction-associated genes. (**D**) The protein levels of MYH7B and β-actin in the hearts were analyzed by Western blot. (**E**) Venn diagram showing the overlapping genes. (**F**) Gene Ontology term enrichment analysis of the overlapping genes identified from E.

study was the use of RPMI 1640 medium for cell culture rather than DMEM, which resulted in less melanin production by the B16F10 cells [36]. While melanoma melanin content is known to impact radiotherapy efficacy [37], whether melanization level affects metastasis propensity requires further investigation.

In patients with cancer cachexia, heart failure and cardiac dysfunction are often significant contributors to cachexia-induced deaths [38,39]. A causal link between the onset of cancer cachexia and cardiac failure, unrelated to anticancer therapy toxicity, has been

widely reported in animal studies [40]. Our data suggests that exercise offers benefits to the heart during metastatic melanoma-induced cancer cachexia in mice. This is in line with previous reports showing that exercise delays cardiac remodeling in mice treated with subcutaneous colon adenocarcinoma cells [41], and in a rat model of mammary tumorigenesis [42]. Unlike prior studies, we used a model of cachexia induced by metastatic melanoma, as the incidence and severity of cachexia increase with metastatic progression [6]. In this model, cardiac functionality was directly assessed by echocardiography, revealing that exercise could notably mitigate the decrease in the heart's systolic function caused by metastatic melanoma. This is contrary to earlier studies suggesting that exercise only tends to improve the heart's systolic function, without achieving statistical significance [41]. Therefore, in our metastatic melanoma-induced cancer cachexia models, we provide compelling evidence that exercise can improve the systolic function of the heart during melanoma-induced cancer cachexia in mice.

Studies of cardiac atrophy in patients with cancer cachexia have indicated that heart weight and left ventricular wall thickness are significantly reduced, accompanied by increased disorganization of myofibrillar proteins and disruption of sarcomere structure, compared with control patients who succumbed to noncancer-related pathologies [40,43,44]. The loss of myofibrillar proteins and cardiomyocytes is thus speculated to be a major contributor to impaired cardiac contractility. In this study, we found that exercise could elevate the decreased expression of MYH7B, a component of myofibrillar proteins [45,46], suggesting that exercise might mitigate the loss of myofibrillar proteins in the heart. Moreover, some key genes previously reported to affect heart failure exhibited significant changes following exercise. For example, CACNB2, which has been negatively associated with heart failure [47], saw its tumor-induced decreased expression reversed after exercise. TMEM65, which is required for connexin43 localization and function in cultured mouse neonatal cardiomyocytes, has been implicated in dilated cardiomyopathy, severe fibrosis, and congestive heart failure when deficient [32,48]. In our study, we found that exercise reversed the decreased expression of Tmem65, indicating potential benefits of exercise in managing cancer-induced heart failure during cardiac cachexia.

Exercise has been shown to benefit cancer survivors by altering disease progression and reducing treatment side effects [16]. However, population-specific evidence for exercise in the context of cancer cachexia is still limited, contributing to a lack of standardized treatments [49]. In our study, low-intensity endurance exercise mitigated cachexia-induced cardiac dysfunction in tumor-bearing mice, consistent with our previous report in tumor-free mice [23]. In clinical practice, baseline exercise testing represents the gold standard for prescribing customized exercise regimens for heart failure patients [50], often involving relatively low intensities [51]. Thus, low-intensity endurance exercise tailored to individual functional capacity may also benefit cardiac health in cachectic patients. However, exercise alone did not rescue cachexia-induced weight loss in our mouse model, indicating combinatorial approaches are likely needed, including exercise, nutrition, pharmacology, and symptom management [52,53].

In conclusion, our results demonstrate that exercise can significantly ameliorate systolic dysfunction induced by metastatic melanoma and can modify the transcriptome of the heart during cancer cachexia in mice. These findings underscore that exercise can offer significant benefits for cardiac function during cancer cachexia.

Ethics statement

Animal experiments were conducted following the guidelines of the Animal Care and Use Committee at Xiamen University (Permit No. XMULAC20200150).

Funding

The work was supported by grants from the National Natural Science Foundation of China, China (grant no. 82000304) and Scientific Research Foundation of the Xiamen Human Resource Ministry for Returned Chinese Scholars (2023) to Liu B; the National Key Research and Development Program of China, China (2019YFE0113900), the Xiamen Medical and Health Key Project (3502Z20204005) and the Fujian Provincial Health Technology Project (2021QNB021) to C. Dai.

Data availability statement

The data set generated in this work was available at GEO: GSE240131. Additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

CRediT authorship contribution statement

Lin Wang: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Xuchao Wang:** Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Jingyu Chen:** Investigation, Formal analysis. **Yang Liu:** Investigation, Formal analysis. **Gang Wang:** Investigation, Formal analysis. **Linjian Chen:** Investigation, Formal analysis. **Wei Ni:** Investigation, Formal analysis. **Yijia Jia:** Writing – review & editing, Writing – original draft. **Cuilian Dai:** Writing – review & editing, Resources, Funding acquisition, Data curation. **Wei Shao:** Visualization, Investigation, Conceptualization. **Binbin Liu:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J.M. Argilés, S. Busquets, B. Stemmler, F.J. López-Soriano, Cancer cachexia: understanding the molecular basis, Nat. Rev. Cancer 14 (2014) 754–762, https://doi.org/10.1038/nrc3829.

[2] V.E. Baracos, L. Martin, M. Korc, D.C. Guttridge, K.C.H. Fearon, Cancer-associated cachexia, Nat Rev Dis Primers 4 (2018) 17105, https://doi.org/10.1038/nrdp.2017.105.

[3] D. Walsh, S. Donnelly, L. Rybicki, The symptoms of advanced cancer: relationship to age, gender, and performance status in 1,000 patients, Support. Care Cancer 8 (2000) 175–179, https://doi.org/10.1007/s005200050281.

[4] A.J. Johnston, K.T. Murphy, L. Jenkinson, D. Laine, K. Emmrich, P. Faou, R. Weston, K.M. Jayatilleke, J. Schloegel, G. Talbo, J.L. Casey, V. Levina, W.W.-L. Wong, H. Dillon, T. Sahay, J. Hoogenraad, H. Anderton, C. Hall, P. Schneider, M. Tanzer, M. Foley, A.M. Scott, P. Gregorevic, S.Y. Liu, L.C. Burkly, G.S. Lynch, J. Silke, N.J. Hoogenraad, Targeting of Fn14 prevents cancer-induced cachexia and Prolongs survival, Cell 162 (2015) 1365–1378, https://doi.org/10.1016/j.cell.2015.08.031.

[5] A.K. Biswas, S. Acharyya, Understanding cachexia in the context of metastatic progression, Nat. Rev. Cancer 20 (2020) 274–284, https://doi.org/10.1038/s41568-020-0251-4.

[6] E. Bruera, C. Sweeney, Cachexia and asthenia in cancer patients, Lancet Oncol. 1 (2000) 138–147, https://doi.org/10.1016/s1470-2045(00)00033-4.

[7] G. Wang, A.K. Biswas, W. Ma, M. Kandpal, C. Coker, P.M. Grandgenett, M.A. Hollingsworth, R. Jain, K. Tanji, S. López-Pintado, A. Borczuk, D. Hebert, S. Jenkitkasemwong, S. Hojyo, R.V. Davuluri, M.D. Knutson, T. Fukada, S. Acharyya, Metastatic cancers promote cachexia through ZIP14 upregulation in skeletal muscle, Nat Med 24 (2018) 770–781, https://doi.org/10.1038/s41591-018-0054-2.

[8] X. Zhou, J.L. Wang, J. Lu, Y. Song, K.S. Kwak, Q. Jiao, R. Rosenfeld, Q. Chen, T. Boone, W.S. Simonet, D.L. Lacey, A.L. Goldberg, H.Q. Han, Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival, Cell 142 (2010) 531–543, https://doi.org/10.1016/j.cell.2010.07.011.

[9] F.A. Graca, A. Stephan, Y.-D. Wang, A. Shirinifard, J. Jiao, P. Vogel, M. Labelle, F. Demontis, Progressive development of melanoma-induced cachexia differentially impacts organ systems in mice, Cell Rep. 42 (2023) 111934, https://doi.org/10.1016/j.celrep.2022.111934.

[10] M.P. Wiggs, A.G. Beaudry, M.L. Law, Cardiac remodeling in cancer-induced cachexia: functional, structural, and metabolic contributors, Cells 11 (2022) 1931, https://doi.org/10.3390/cells11121931.

[11] S.M.R. Kazemi-Bajestani, H. Becher, C. Butts, N.S. Basappa, M. Smylie, A.A. Joy, R. Sangha, A. Gallivan, P. Kavsak, Q. Chu, V.E. Baracos, Rapid atrophy of cardiac left ventricular mass in patients with non-small cell carcinoma of the lung, J Cachexia Sarcopenia Muscle 10 (2019) 1070–1082, https://doi.org/10.1002/jcsm.12451.

[12] V. Rausch, V. Sala, F. Penna, P.E. Porporato, A. Ghigo, Understanding the common mechanisms of heart and skeletal muscle wasting in cancer cachexia, Oncogenesis 10 (2021) 1–13, https://doi.org/10.1038/s41389-020-00288-6.

[13] A.J. Grande, V. Silva, R. Riera, A. Medeiros, S.G.P. Vitoriano, M.S. Peccin, M. Maddocks, Exercise for cancer cachexia in adults, Cochrane Database Syst. Rev. (2014) CD010804, https://doi.org/10.1002/14651858.CD010804.pub2.

[14] M. Maddocks, A.J. Murton, A. Wilcock, Therapeutic exercise in cancer cachexia, CRO 17 (2012), https://doi.org/10.1615/CritRevOncog.v17.i3.60.

[15] I.-M. Lee, E.J. Shiroma, F. Lobelo, P. Puska, S.N. Blair, P.T. Katzmarzyk, Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy, Lancet 380 (2012) 219–229, https://doi.org/10.1016/S0140-6736(12)61031-9.

[16] K.H. Schmitz, K.S. Courneya, C. Matthews, W. Demark-Wahnefried, D.A. Galvão, B.M. Pinto, M.L. Irwin, K.Y. Wolin, R.J. Segal, A. Lucia, C.M. Schneider, V. E. von Gruenigen, A.L. Schwartz, American College of Sports medicine, American College of Sports medicine roundtable on exercise guidelines for cancer survivors, Med. Sci. Sports Exerc. 42 (2010) 1409–1426, https://doi.org/10.1249/MSS.0b013e3181e0c112.

[17] G. Mavropalias, M. Sim, D.R. Taaffe, D.A. Galvão, N. Spry, W.J. Kraemer, K. Häkkinen, R.U. Newton, Exercise medicine for cancer cachexia: targeted exercise to counteract mechanisms and treatment side effects, J. Cancer Res. Clin. Oncol. 148 (2022) 1389–1406, https://doi.org/10.1007/s00432-022-03927-0.

[18] S. Tsitkanou, K.A. Murach, T.A. Washington, N.P. Greene, Exercise counteracts the Deleterious effects of cancer cachexia, Cancers 14 (2022) 2512, https://doi.org/10.3390/cancers14102512.

[19] Y. Belloum, F. Rannou-Bekono, F.B. Favier, Cancer-induced cardiac cachexia: Pathogenesis and impact of physical activity, Oncol. Rep. 37 (2017) 2543–2552, https://doi.org/10.3892/or.2017.5542.

[20] M.A. Nystoriak, A. Bhatnagar, Cardiovascular effects and benefits of exercise, Front Cardiovasc Med 5 (2018) 135, https://doi.org/10.3389/fcvm.2018.00135.

[21] M. Gleeson, N.C. Bishop, D.J. Stensel, M.R. Lindley, S.S. Mastana, M.A. Nimmo, The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease, Nat. Rev. Immunol. 11 (2011) 607–615, https://doi.org/10.1038/nri3041.

[22] V. Bora, B.M. Patel, Investigation into the role of anti-diabetic agents in cachexia associated with metastatic cancer, Life Sci. 274 (2021) 119329, https://doi.org/10.1016/j.lfs.2021.119329.

[23] G. Wang, L. Wang, X. Wang, H. Ye, W. Ni, W. Shao, C. Dai, B. Liu, Low-intensity exercise training increases systolic function of heart and MHCII low cardiac resident macrophages, Heliyon 9 (2023) e22915, https://doi.org/10.1016/j.heliyon.2023.e22915.

[24] Z. Li, Z. Wang, T. Sun, S. Liu, S. Ding, L. Sun, Identifying key genes in CD4+ T cells of systemic lupus erythematosus by integrated bioinformatics analysis, Front. Genet. 13 (2022) 941221, https://doi.org/10.3389/fgene.2022.941221.

[25] D.-L. Guo, Z.-G. Wang, M.-S. Pei, L.-L. Guo, Y.-H. Yu, Transcriptome analysis reveals mechanism of early ripening in Kyoho grape with hydrogen peroxide treatment, BMC Genom. 21 (2020) 784, https://doi.org/10.1186/s12864-020-07180-y.

[26] B. Liu, T. Maekawa, K. Yoshida, N.H. Ly, K. Inoue, A. Hasegawa, B. Chatton, A. Ogura, S. Ishii, Telomere shortening by transgenerational transmission of TNF-α-induced TERRA via ATF7, Nucleic Acids Res. 47 (2019) 283–298, https://doi.org/10.1093/nar/gky1149.

[27] R.-Y. Xu, X.-F. Zhu, Y. Yang, P. Ye, High-sensitive cardiac troponin T, J Geriatr Cardiol 10 (2013) 102–109, https://doi.org/10.3969/j.issn.1671-5411.2013.01.015.

[28] D.A. Morrow, C.P. Cannon, R.L. Jesse, L.K. Newby, J. Ravkilde, A.B. Storrow, A.H.B. Wu, R.H. Christenson, National Academy of clinical Biochemistry, National Academy of clinical Biochemistry Laboratory medicine practice guidelines: clinical characteristics and utilization of biochemical markers in acute coronary syndromes, Circulation 115 (2007) e356–e375, https://doi.org/10.1161/CIRCULATIONAHA.107.182882.

[29] P. Chen, Z. Li, J. Nie, H. Wang, B. Yu, Z. Wen, Y. Sun, X. Shi, L. Jin, D.-W. Wang, MYH7B variants cause hypertrophic cardiomyopathy by activating the CaMK-signaling pathway, Sci. China Life Sci. 63 (2020) 1347–1362, https://doi.org/10.1007/s11427-019-1627-y.

[30] S. Beca, P.B. Helli, J.A. Simpson, D. Zhao, G.P. Farman, P. Jones, X. Tian, L.S. Wilson, F. Ahmad, S.R.W. Chen, M.A. Movsesian, V. Manganiello, D.H. Maurice, M. Conti, P.H. Backx, Phosphodiesterase 4D (PDE4D) regulates baseline sarcoplasmic reticulum Ca2+ release and cardiac contractility, independently of L-type Ca2+current, Circ. Res. 109 (2011) 1024–1030, https://doi.org/10.1161/CIRCRESAHA.111.250464.

[31] K. Roder, A.A. Werdich, W. Li, M. Liu, T.Y. Kim, L.E. Organ-Darling, K.S. Moshal, J.M. Hwang, Y. Lu, B.-R. Choi, C.A. MacRae, G. Koren, RING finger protein RNF207, a novel regulator of cardiac excitation, J. Biol. Chem. 289 (2014) 33730–33740, https://doi.org/10.1074/jbc.M114.592295.

[32] A.C.T. Teng, L. Gu, M. Di Paola, R. Lakin, Z.J. Williams, A. Au, W. Chen, N.I. Callaghan, F.H. Zadeh, Y.-Q. Zhou, M. Fatah, D. Chatterjee, L.J. Jourdan, J. Liu, C. A. Simmons, T. Kislinger, C.M. Yip, P.H. Backx, R.G. Gourdie, R.M. Hamilton, A.O. Gramolini, Tmem65 is critical for the structure and function of the intercalated discs in mouse hearts, Nat. Commun. 13 (2022) 6166, https://doi.org/10.1038/s41467-022-33303-y.

[33] Q. Aziz, Y. Li, A. Tinker, Potassium channels in the sinoatrial node and their role in heart rate control, Channels 12 (2018) 356–366, https://doi.org/10.1080/19336950.2018.1532255.
[34] A. Carpinteiro, K.A. Becker, L. Japtok, G. Hessler, S. Keitsch, M. Požgajovà, K.W. Schmid, C. Adams, S. Müller, B. Kleuser, M.J. Edwards, H. Grassmé, I. Helfrich, E. Gulbins, Regulation of hematogenous tumor metastasis by acid sphingomyelinase, EMBO Mol. Med. 7 (2015) 714–734, https://doi.org/10.15252/emmm.201404571.
[35] C.-H. Kuo, Y.-F. Wu, B.-I. Chang, C.-K. Hsu, C.-H. Lai, H.-L. Wu, Interference in melanoma CD248 function reduces vascular mimicry and metastasis, J. Biomed. Sci. 29 (2022) 98, https://doi.org/10.1186/s12929-022-00882-3.
[36] A. Skoniecka, M. Cichorek, A. Tyminska, I. Pelikant-Malecka, J. Dziewiatkowski, Melanization as unfavorable factor in amelanotic melanoma cell biology, Protoplasma 258 (2021) 935–948, https://doi.org/10.1007/s00709-021-01613-5.
[37] A.A. Brożyna, W. Jóźwicki, K. Roszkowski, J. Filipiak, A.T. Slominski, Melanin content in melanoma metastases affects the outcome of radiotherapy, Oncotarget 7 (2016) 17844–17853, https://doi.org/10.18632/oncotarget.7528.
[38] A. Barkhudaryan, N. Scherbakov, J. Springer, W. Doehner, Cardiac muscle wasting in individuals with cancer cachexia, ESC Heart Fail 4 (2017) 458–467, https://doi.org/10.1002/ehf2.12184.
[39] N. Pavo, M. Raderer, M. Hülsmann, S. Neuhold, C. Adlbrecht, G. Strunk, G. Goliasch, H. Gisslinger, G.G. Steger, M. Hejna, W. Köstler, S. Zöchbauer-Müller, C. Marosi, G. Kornek, L. Auerbach, S. Schneider, B. Parschalk, W. Scheithauer, R. Pirker, J. Drach, C. Zielinski, R. Pacher, Cardiovascular biomarkers in patients with cancer and their association with all-cause mortality, Heart 101 (2015) 1874–1880, https://doi.org/10.1136/heartjnl-2015-307848.
[40] J. Springer, A. Tschirner, A. Haghikia, S. von Haehling, H. Lal, A. Grzesiak, E. Kaschina, S. Palus, M. Pötsch, K. von Websky, B. Hocher, C. Latouche, F. Jaisser, L. Morawietz, A.J.S. Coats, J. Beadle, J.M. Argiles, T. Thum, G. Földes, W. Doehner, D. Hilfiker-Kleiner, T. Force, S.D. Anker, Prevention of liver cancer cachexia-induced cardiac wasting and heart failure, Eur. Heart J. 35 (2014) 932–941, https://doi.org/10.1093/eurheartj/eht302.
[41] L.G. Fernandes, G.C. Tobias, A.O. Paixão, P.M. Dourado, V.A. Voltarelli, P.C. Brum, Exercise training delays cardiac remodeling in a mouse model of cancer cachexia, Life Sci. 260 (2020) 118392, https://doi.org/10.1016/j.lfs.2020.118392.
[42] A.I. Padrão, D. Moreira-Gonçalves, P.A. Oliveira, C. Teixeira, A.I. Faustino-Rocha, L. Helguero, R. Vitorino, L.L. Santos, F. Amado, J.A. Duarte, R. Ferreira, Endurance training prevents TWEAK but not myostatin-mediated cardiac remodelling in cancer cachexia, Arch. Biochem. Biophys. 567 (2015) 13–21, https://doi.org/10.1016/j.abb.2014.12.026.
[43] M. Tian, Y. Nishijima, M.L. Asp, M.B. Stout, P.J. Reiser, M.A. Belury, Cardiac alterations in cancer-induced cachexia in mice, Int. J. Oncol. 37 (2010) 347–353, https://doi.org/10.3892/ijo_00000683.
[44] J.M.M. Antunes, R.M.P. Ferreira, D. Moreira-Gonçalves, Exercise training as therapy for cancer-induced cardiac cachexia, Trends Mol. Med. 24 (2018) 709–727, https://doi.org/10.1016/j.molmed.2018.06.002.
[45] K. Ojima, Myosin: Formation and maintenance of thick filaments, Anim. Sci. J. 90 (2019) 801–807, https://doi.org/10.1111/asj.13226.
[46] A.K. Peter, A.C. Rossi, M. Buvoli, C.D. Ozeroff, C. Crocini, A.R. Perry, A.E. Buvoli, L.A. Lee, L.A. Leinwand, Expression of normally repressed myosin Heavy Chain 7b in the Mammalian heart induces dilated cardiomyopathy, J. Am. Heart Assoc. 8 (2019) e013318, https://doi.org/10.1161/JAHA.119.013318.
[47] Y. Chernyavskaya, A.M. Ebert, E. Milligan, D.M. Garrity, Voltage-gated calcium channel CACNB2 (β2.1) protein is required in the heart for control of cell proliferation and heart tube integrity, Dev. Dynam. 241 (2012) 648–662, https://doi.org/10.1002/dvdy.23746.
[48] P. Sharma, C. Abbasi, S. Lazic, A.C.T. Teng, D. Wang, N. Dubois, V. Ignatchenko, V. Wong, J. Liu, T. Araki, M. Tiburcy, C. Ackerley, W.H. Zimmermann, R. Hamilton, Y. Sun, P.P. Liu, G. Keller, I. Stagljar, I.C. Scott, T. Kislinger, A.O. Gramolini, Evolutionarily conserved intercalated disc protein Tmem65 regulates cardiac conduction and connexin 43 function, Nat. Commun. 6 (2015) 8391, https://doi.org/10.1038/ncomms9391.
[49] C. Cheung, E. Boocock, A.J. Grande, M. Maddocks, Exercise-based interventions for cancer cachexia: a systematic review of randomised and non-randomised controlled trials, Asia-Pacific Journal of Oncology Nursing (2023) 100335, https://doi.org/10.1016/j.apjon.2023.100335.
[50] M. Mytinger, R.K. Nelson, M. Zuhl, Exercise Prescription guidelines for cardiovascular disease patients in the Absence of a baseline stress test, J Cardiovasc Dev Dis 7 (2020) 15, https://doi.org/10.3390/jcdd7020015.
[51] R. Belardinelli, D. Georgiou, V. Scocco, T.J. Barstow, A. Purcaro, Low intensity exercise training in patients with chronic heart failure, J. Am. Coll. Cardiol. 26 (1995) 975–982, https://doi.org/10.1016/0735-1097(95)00267-1.
[52] M. Saeteaw, P. Sanguanboonyaphong, J. Yoodee, K. Craft, R. Sawangjit, N. Ngamphaiboon, P.C. Shantavasinkul, S. Subongkot, N. Chaiyakunapruk, Efficacy and safety of pharmacological cachexia interventions: systematic review and network meta-analysis, BMJ Support. Palliat. Care 11 (2021) 75–85, https://doi.org/10.1136/bmjspcare-2020-002601.
[53] S. Miura, T. Naito, S. Mitsunaga, K. Omae, K. Mori, T. Inano, T. Yamaguchi, N. Tatematsu, T. Okayama, A. Morikawa, T. Mouri, H. Tanaka, M. Kimura, H. Imai, T. Mizukami, A. Imoto, C. Kondoh, S. Shiotsu, H. Okuyama, M. Ueno, T. Takahashi, T. Tsuji, H. Aragane, A. Inui, T. Higashiguchi, K. Takayama, A randomized phase II study of nutritional and exercise treatment for elderly patients with advanced non-small cell lung or pancreatic cancer: the NEXTAC-TWO study protocol, BMC Cancer 19 (2019) 528, https://doi.org/10.1186/s12885-019-5762-6.