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ORIGINAL RESEARCH ARTICLE

Endurance training attenuates TNF-α elevation in a murine model of lung adenocarcinoma cachexia: A pilot study

Naoki Maki[1,2]*, Takahiro Yanagihara[2], Kazuto Sugai[2], Tomoyuki Kawamura[2], Yusuke Saeki[2], Naohiro Kobayashi[2], Harumi Sakamoto[1], Keisuke Taniguchi[1], Hideo Ichimura[2], and Yukio Sato[2]

[1]Department of Rehabilitation, Faculty of Rehabilitation, R Professional University of Rehabilitation, Tsuchiura, Ibaraki, Japan

[2]Department of Thoracic Surgery, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

Abstract

Introduction: Cancer cachexia is a multifactorial syndrome characterized by weight loss, skeletal muscle wasting, and systemic inflammation. Tumor necrosis factor-alpha (TNF-α) plays a central role in cachexia pathophysiology; however, the anti-inflammatory effects of endurance exercise in lung adenocarcinoma–associated cachexia remain insufficiently defined.

Objective: This study aimed to investigate the effects of endurance training on systemic inflammation, as assessed by circulating TNF-α levels, and cachexia-related outcomes in a murine orthotopic model of lung adenocarcinoma.

Methods: Male BALB/cAJcl-nu/nu mice were assigned to four groups: non-exercise control, exercise control, non-exercise lung adenocarcinoma cachexia, and exercise lung adenocarcinoma cachexia (n = 6 per group). An orthotopic lung adenocarcinoma model was established by trans-airway implantation of A549 cells. Endurance training was performed at 20 m/min for 30 min, five days per week for six weeks. Body weight, gastrocnemius wet weight, circulating TNF-α levels, and lung macroscopic and histological findings were evaluated. Data were analyzed using one-way and two-way analyses of variance.

Results: After six weeks, tumor-bearing mice exhibited significant body weight loss and skeletal muscle atrophy compared with controls, confirming successful induction of cachexia. Endurance training partially attenuated body weight loss in tumor-bearing mice. Macroscopic and histological analyses confirmed lung tumor presence in both non-exercise and exercise cachexia groups. Circulating TNF-α levels were significantly elevated in tumor-bearing mice compared with controls; notably, endurance training significantly reduced TNF-α levels in cachexia mice. Two-way analysis revealed significant main effects of tumor burden and exercise on TNF-α levels.

Conclusion: Endurance training attenuates systemic TNF-α elevation in a murine model of lung adenocarcinoma cachexia without suppressing tumor establishment. These findings support endurance exercise as a supportive anti-inflammatory strategy in cancer cachexia.

Keywords: Lung adenocarcinoma; Cancer cachexia; Endurance exercise; TNF-α; Inflammation

***Corresponding author:**
Naoki Maki
(maki@u.a-ru.ac.jp)

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1. Introduction

Cancer cachexia is a multifactorial metabolic syndrome characterized by involuntary body weight loss, skeletal muscle wasting, and chronic systemic inflammation, and it is frequently observed in patients with advanced malignancies.[1-3] Unlike starvation, cancer cachexia cannot be fully reversed by nutritional support alone and is strongly associated with reduced treatment tolerance, impaired physical function, and poor prognosis.[3,4]

Lung cancer remains the leading cause of cancer-related mortality worldwide, with lung adenocarcinoma representing the most prevalent histological subtype.[5] Cachexia is particularly common in lung adenocarcinoma because tumor-induced metabolic alterations, systemic inflammation, and respiratory burden collectively accelerate physical decline.[6,7] Among inflammatory mediators, tumor necrosis factor-alpha (TNF-α) plays a central role in the pathophysiology of cachexia by promoting skeletal muscle proteolysis, mitochondrial dysfunction, and catabolic signaling cascades.[8-10]

Current clinical guidelines emphasize the importance of multimodal approaches for cachexia management, integrating pharmacological treatment, nutritional support, and exercise therapy.[11] Exercise training has attracted increasing attention as a non-pharmacological intervention capable of modulating systemic inflammation and metabolic homeostasis.[12-14] Preclinical studies suggest that endurance exercise can attenuate cachexia-related muscle wasting and inflammatory responses in tumor-bearing animals.[15,16] However, evidence focusing on inflammatory modulation in lung adenocarcinoma–associated cachexia remains limited.

Moreover, many experimental cachexia studies rely on subcutaneous tumor models, which fail to replicate lung-specific tumor–host interactions.[17] In contrast, orthotopic lung cancer models provide a more clinically relevant platform for evaluating the systemic effects of tumor burden and supportive interventions.

Therefore, this expanded pilot study aimed to investigate the effects of endurance training on systemic inflammation, with a particular focus on circulating TNF-α, using an orthotopic murine model of lung adenocarcinoma cachexia. We hypothesized that endurance exercise would attenuate inflammation in tumor-bearing mice without suppressing tumor establishment.

2. Materials and methods

2.1. Study design

This study was conducted as a preclinical interventional pilot study using a murine model of lung adenocarcinoma cachexia. Mice were allocated into four groups: a non-exercise control group, an exercise control group, a non-exercise lung adenocarcinoma cachexia group, and an exercise lung adenocarcinoma cachexia group. Group comparisons were performed to explore the effects of exercise intervention on cachexia-related outcomes. The study was conducted from January 2022 to July 2024.

2.2. Experimental animals

Male BALB/cAJcl-nu/nu mice (9 weeks old) were purchased from CLEA Japan, Inc. (Japan) and housed under controlled environmental conditions (12-h light–dark cycle, 22–24 °C) with *ad libitum* access to standard laboratory chow and water. Animals were monitored daily for general health status and signs of distress. All experimental procedures were approved by the Animal Experiment Committee of the University of Tsukuba (approval number: 21-461) and were conducted in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0 guidelines.[18] To ensure animal welfare, predefined humane endpoints were established prior to the experiment. Mice were closely monitored for signs of distress, including marked body weight loss (>20% from baseline), reduced mobility, decreased food and water intake, and abnormal posture or appearance. Animals meeting these criteria were humanely euthanized to minimize suffering. All procedures were conducted in accordance with institutional guidelines and ARRIVE 2.0 recommendations.

2.3. Lung adenocarcinoma cachexia model

An orthotopic murine model of lung adenocarcinoma–associated cachexia was established using a trans-airway implantation approach optimized for pulmonary tumor localization. Human lung adenocarcinoma A549 cells were maintained under standard culture conditions (37 °C, 5% CO_2) and collected during the exponential growth phase. The A549 cells were purchased from the RIKEN BioResource Research Center (RIKEN BRC), Tsukuba, Japan. Cells were rinsed with phosphate-buffered saline and resuspended at a final concentration of 1×10^7 cells/mL prior to implantation.

Mice were anesthetized with isoflurane and positioned supine with gentle cervical extension to facilitate airway access. A small midline skin incision was made at the lower cervical region to visualize the trachea. A 24-gauge catheter was carefully introduced between the tracheal cartilaginous rings and advanced toward the distal airway. Subsequently, 0.1 mL of the A549 cell suspension (1×10^6 cells per mouse) was slowly administered intratracheally. After cell delivery, the catheter was withdrawn, and the

incision was closed using standard suturing techniques. Animals were returned to their cages after complete recovery from anesthesia.

This orthotopic implantation strategy induces tumor growth confined to the lung parenchyma, thereby reproducing lung-specific tumor–host interactions and their systemic consequences. Throughout the experimental period, animals were regularly observed for changes in physical appearance and body weight as indicators of cachexia progression. Humane endpoints were applied in accordance with institutional animal welfare guidelines when predefined criteria were met.

2.4. Exercise intervention

Mice assigned to the exercise groups underwent endurance training using a motorized running wheel system (SN450, Shinano Manufacturing Co., Japan) at a speed of 20 m/min for 30 minutes, once daily, five days per week for six weeks. Before the formal intervention, mice were familiarized with the running wheel to minimize stress-related effects. Non-exercise mice were maintained under identical housing conditions without exercise exposure. This protocol was designed to provide moderate-intensity endurance stimulation while minimizing excessive physiological stress.

2.5. Outcome measures

Body weight was measured weekly throughout the experimental period using a calibrated digital scale (KD-192, Tanita Co., Japan), and body weight at 15 weeks of age was used for statistical analysis to evaluate cachexia-related weight loss.

At the time of sacrifice, bilateral gastrocnemius muscles were carefully excised, and wet muscle weight was measured using an analytical balance (A&D Co., Ltd., Japan) to assess skeletal muscle mass as an indicator of cachexia-associated muscle atrophy.

Blood samples were collected at sacrifice under deep anesthesia and processed according to standard procedures. Circulating tumor necrosis factor-alpha (TNF-α) concentrations were quantified using a high-sensitivity cytokine assay based on a validated multiplex immunoassay platform (Funakoshi Co., Ltd., Japan), following the manufacturer's instructions. This assay enables sensitive and quantitative detection of low-abundance inflammatory cytokines. TNF-α levels were analyzed as a primary outcome measure reflecting systemic inflammation associated with lung adenocarcinoma-induced cachexia and the effects of endurance training.

2.6. Histological evaluation of lung tissue

Gross external appearance of the mice was documented photographically prior to tissue collection (Figure 1). Excised lungs were photographed to evaluate macroscopic tumor findings (Figure 2). Lung tissues were fixed in 10% neutral-buffered formalin, processed for paraffin embedding, and sectioned for hematoxylin and eosin staining. Histological assessment was conducted in a blinded manner at × 200 magnification to verify tumor presence (Figure 3).

2.7. Statistical analysis

All data are expressed as mean ± standard deviation. Group comparisons were performed using one-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. To examine the main and interaction effects of tumor burden and exercise, a two-way ANOVA was applied. A p-value < 0.05 was considered statistically significant. All analyses were conducted using SPSS version 27 (IBM Corporation, Tokyo, Japan).

3. Results

3.1. Body weight

Representative images of overall body appearance are shown in Figure 1.

At 15 weeks of age, body weight was significantly lower in non-exercise cachexia mice (26.71 ± 1.17 g) and exercise cachexia mice (29.08 ± 1.12 g) compared with non-exercise control mice (32.28 ± 0.70 g) and exercise control mice (31.73 ± 1.40 g) (p = 0.003 and p = 0.008, respectively; Table 1). Endurance training partially attenuated body weight loss in tumor-bearing mice, as reflected by significantly higher body weight in exercise cachexia mice compared with non-exercise cachexia mice (29.08 ± 1.12 g versus 26.71 ± 1.17 g, p = 0.021). In contrast, no significant difference was observed between exercised and non-exercised control mice (31.73 ± 1.40 g vs. 32.28 ± 0.70 g, p = 0.412). Longitudinal analysis demonstrated progressive body weight loss in tumor-bearing mice, whereas endurance training partially attenuated this decline over time (Figure 4).

3.2. Macroscopic evaluation of excised lungs

Macroscopic evaluation of excised lungs (Figure 2) revealed normal lung morphology without visible tumor lesions in control mice. In contrast, both non-exercise and exercise cachexia mice exhibited apparent macroscopic tumor lesions within the lung parenchyma, confirming successful tumor establishment irrespective of exercise intervention.

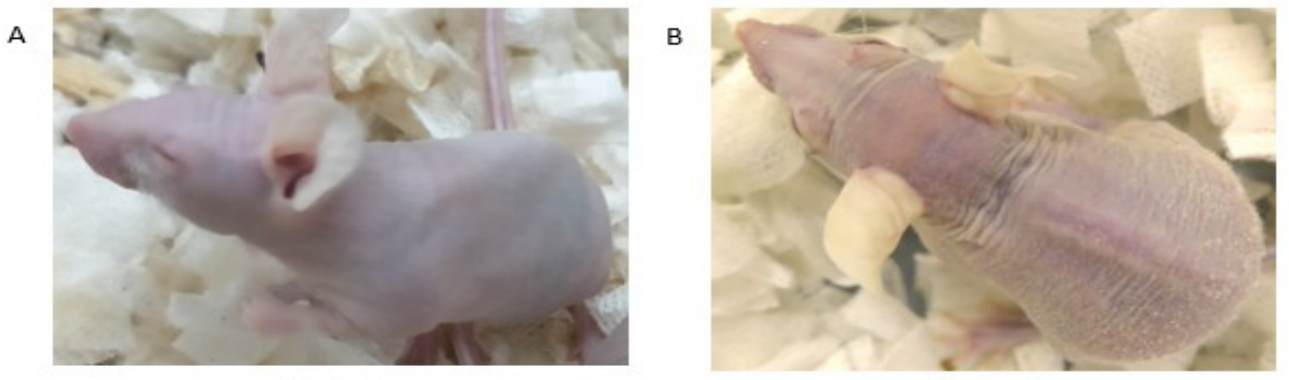

Figure 1. Representative images of overall body appearance. Tumor-bearing mice exhibited marked body weight loss and an emaciated phenotype compared with control mice, indicating successful induction of cachexia. (A) Control. (B) Tumor-bearing mouse.

Table 1. General characteristics of mice

Parameters	Control (*n* = 6)	Exercise (*n* = 6)	Non-exercise cachexia (*n* = 6)	Exercise cachexia (*n* = 6)	C versus NEC	C versus EC	E versus NEC	E versus EC	C versus E	NEC versus EC
Body weight (g/9 w)	26.28 ± 1.56	26.51 ± 1.37	27.08 ± 1.34	26.70 ± 1.67	ns	ns	ns	ns	ns	ns
Body weight (g/15 w)	32.28 ± 0.70	31.73 ± 1.40	26.71 ± 1.17	29.08 ± 1.12	**	**	**	**	ns	**
Gastrocnemius muscle wet weight (g)	1.03 ± 0.41	1.24 ± 0.19	0.11 ± 0.04	0.56 ± 0.58	**	ns	**	*	ns	ns
RBC (μL/ 15w)	557.5 ± 274.74	632.15 ± 166.54	622.34 ± 278.67	625.57 ± 275.18	ns	ns	ns	ns	ns	ns
HGB (g/dL/15 w)	10.91 ± 4.17	13.75 ± 2.47	9.76 ± 4.20	9.82 ± 4.97	ns	ns	ns	ns	ns	ns
WBC (μL/15 w)	1,005.05 ± 477.19	2,640.15 ± 1,062.73	4,850.83 ± 2,958.21	2,383.30 ± 2,338.73	*	ns	ns	ns	ns	ns
PLT (μL/15 w)	5.90 ± 3.67	35.91 ± 1.00	65.80 ± 34.56	8.65 ± 1.71	**	ns	*	ns	*	ns
TNF-α (pg/mL/15 w)	6.70 ± 7.64	5.96 ± 0.49	20.89 ± 1.01	13.75 ± 0.89	**	*	**	*	ns	*

Notes: Data are presented as means and standard deviation.* denotes $p < 0.05$; ** denotes $p < 0.01$; ns: not significant
Abbreviations: C: Control; EC: Exercise cachexia; HGB: Hemoglobin; NEC: Non-exercise cachexia; PLT: Platelet; RBC: Red blood cell; TNF-α: Tumor necrosis factor-alpha; WBC: White blood cell.

3.3. Histological findings

Histological findings are presented in **Figure 4**. Lung sections from control mice showed preserved alveolar architecture without evidence of malignancy. In contrast, lung tissues from both non-exercise and exercise cachexia mice demonstrated characteristic adenocarcinoma features, including dense tumor cell proliferation and disruption of normal alveolar structures.

3.4. Gastrocnemius muscle wet weight

Gastrocnemius muscle wet weight was significantly reduced in cachexia groups compared with controls,

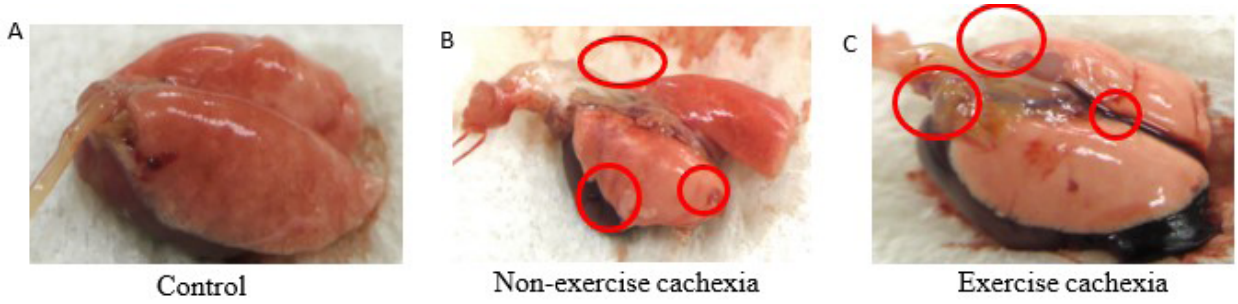

Figure 2. Macroscopic evaluation of excised lungs. Control mice showed normal lung morphology without visible tumor lesions. In contrast, both non-exercise and exercise cachexia mice exhibited apparent macroscopic tumor lesions within the lung parenchyma, confirming successful tumor establishment irrespective of exercise intervention. (A) Control. (B) Non-exercise cachexia. (C) Exercise cachexia.

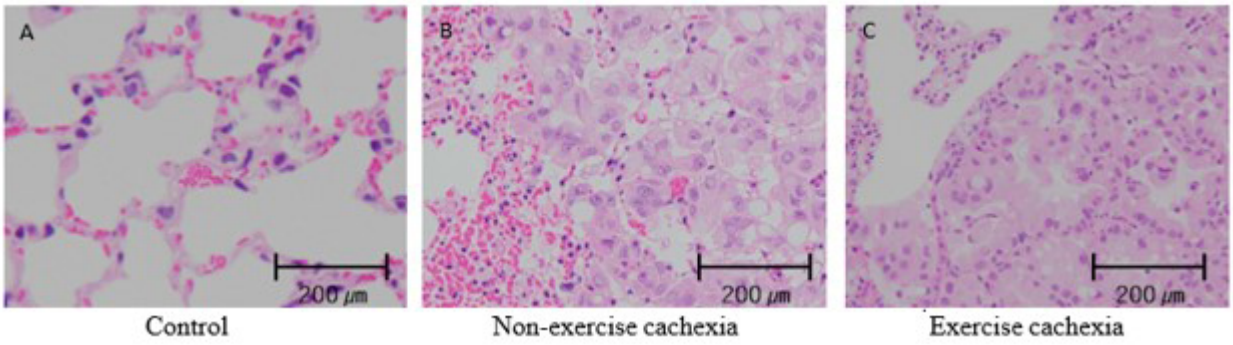

Figure 3. Evaluation of histopathological characteristics of lungs from mice. Representative hematoxylin and eosin-stained lung sections from (A) control, (B) non-exercise cachexia, and (C) exercise cachexia groups. Control mice showed preserved alveolar architecture, whereas cachexia mice exhibited dense tumor cell proliferation and disruption of normal alveolar structures. Images were obtained at × 200 magnification. Scale bar = 200 μm.

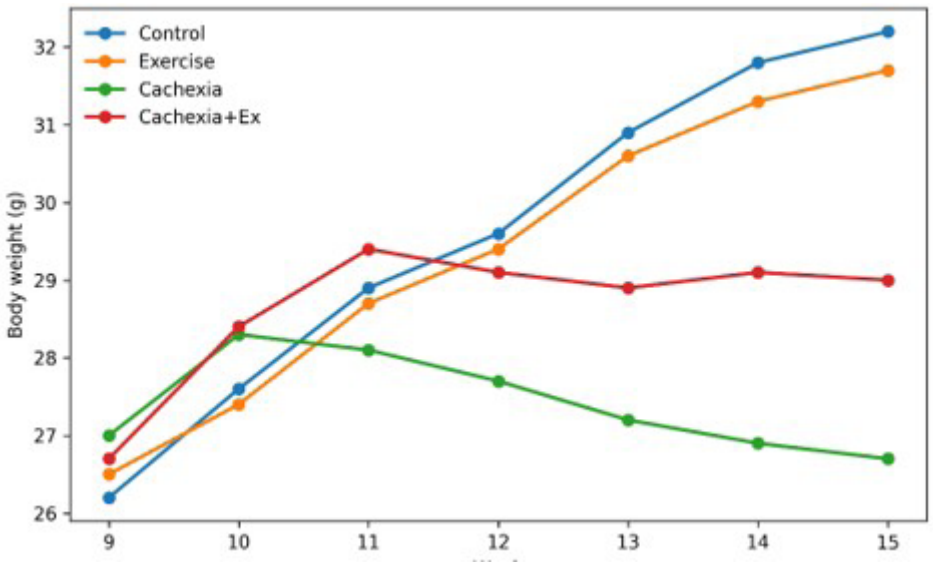

Figure 4. Body weight progression during the experimental period. Changes in body weight from 9 to 15 weeks in the control, exercise, non-exercise cachexia, and exercise cachexia groups. Tumor-bearing mice showed progressive weight loss over time, whereas endurance training partially attenuated this decline in the exercise cachexia group.

indicating cachexia-associated skeletal muscle atrophy (Table 1). Specifically, gastrocnemius muscle weight was markedly lower in non-exercise cachexia mice (0.11 ± 0.04 g) and exercise cachexia mice (0.56 ± 0.58 g) compared with non-exercise control mice (1.03 ± 0.41 g) and exercise control mice (1.24 ± 0.19 g) (p = 0.002 and p = 0.009, respectively). Although exercised cachexia mice tended to exhibit higher muscle weight than non-exercised cachexia mice, this difference did not reach statistical significance (p = 0.087).

3.5. Tumor necrosis factor-alpha levels

Circulating TNF-α levels at 15 weeks were significantly elevated in tumor-bearing mice compared with controls (non-exercise cachexia versus control: p = 0.001; exercise cachexia versus control: p = 0.018; Table 1, Figure 5). Endurance training significantly reduced TNF-α levels in cachexia mice compared with non-exercised cachexia mice (13.75 ± 0.89 versus 20.89 ± 1.01 pg/mL, p = 0.012). Two-way analysis of variance (Table 2) demonstrated a significant main effect of tumor burden on TNF-α levels (F = 47.82, p < 0.001, partial η^2 = 0.71) as well as a significant main effect of exercise (F = 6.14, p = 0.022, partial η^2 = 0.23). Notably, endurance training significantly reduced TNF-α levels in cachexia mice compared with non-exercised cachexia mice. Although the interaction between tumor burden and exercise did not reach statistical significance (F = 4.06, p = 0.058), a trend toward interaction was observed, suggesting that endurance training modulates systemic inflammation predominantly in the presence of tumor-induced cachexia.

4. Discussion

This expanded pilot study demonstrates that endurance training attenuates systemic inflammation, as evidenced by reduced circulating TNF-α levels, in a murine model of lung adenocarcinoma cachexia. Importantly, this anti-inflammatory effect occurred without suppression of tumor establishment, as confirmed by macroscopic and histological lung findings.

Tumor necrosis factor-alpha is a central mediator of cancer cachexia, promoting skeletal muscle proteolysis, mitochondrial dysfunction, and metabolic derangements.[8–10,19] Importantly, the role of TNF-α as a key driver of systemic inflammation in cancer cachexia is already well established in both experimental and clinical studies. Elevated TNF-α levels are associated with body weight loss, functional decline, and poor prognosis in patients with lung cancer.[6,7] The present findings are

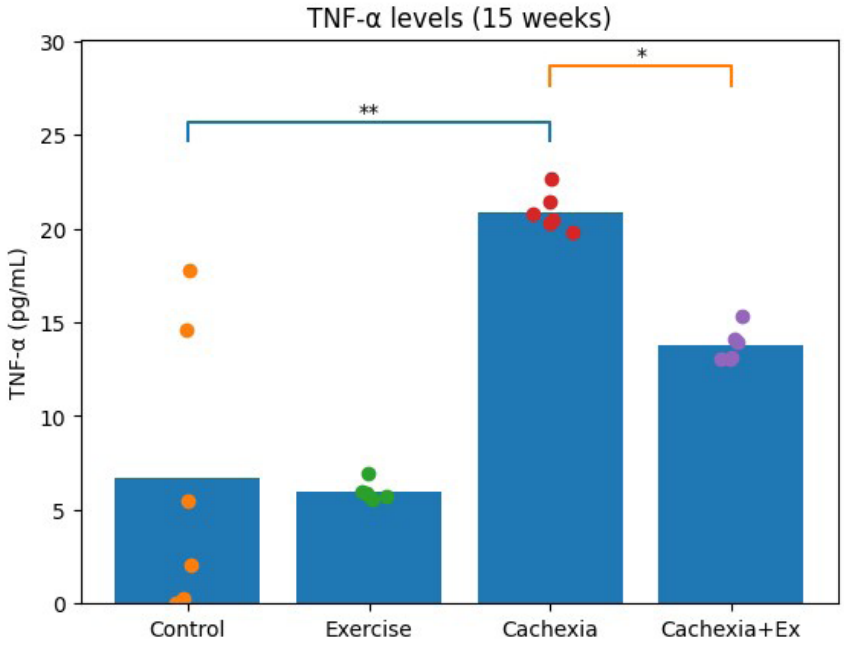

Figure 5. Effects of endurance training on circulating tumor necrosis factor-alpha (TNF-α) levels. Circulating TNF-α levels at 15 weeks in control, exercise, non-exercise cachexia, and exercise cachexia groups were shown. Data are presented as boxplots with individual data points (n = 6 per group). Tumor-bearing mice exhibited significantly elevated TNF-α levels compared with controls, while endurance training significantly reduced TNF-α levels in cachexia mice. Two-way ANOVA revealed significant main effects of tumor burden (p < 0.001) and exercise (p = 0.022), with no significant interaction (p = 0.057). * denotes p < 0.05, ** denotes p < 0.01.

Table 2. Two-way analysis of variance of circulating TNF-α levels at 15 weeks

Outcome	df	*F*	*p*	Partial η^2
TNF-α (pg/mL/15 w) tumor	1	47.824	<0.001**	0.705
TNF-α (pg/mL/15 w) exercise	1	6.137	0.022*	0.234
TNF-α (pg/mL/15 w) tumor × exercise	1	4.057	0.057	0.168
Body weight (g/15 w) tumor	1	79.060	<0.001**	0.798
Body weight (g/15 w) exercise	1	3.864	0.063	0.161
Body weight (g/15 w) tumor × exercise	1	9.961	0.004**	0.332
Gastrocnemius muscle wet weight (g) tumor	1	28.713	<0.001**	0.589
Gastrocnemius muscle wet weight (g) exercise	1	5.111	0.035*	0.203
Gastrocnemius muscle wet weight (g) tumor × exercise	1	0.762	0.393	0.036

Notes: Data are presented as mean and standard deviation.* denotes $p < 0.05$, ** denotes $p < 0.01$. Tumor indicates tumor burden (control versus cachexia), and exercise indicates endurance training (no exercise versus exercise). Partial eta squared (η^2) represents effect size.
Abbreviation: TNF-α: Tumor necrosis factor-alpha.

consistent with these clinical observations and suggest that endurance exercise can modulate systemic inflammation even in the presence of established lung tumors.

Previous experimental studies have reported beneficial effects of endurance exercise on cachexia-related outcomes; however, many relied on subcutaneous tumor models.[15,16] By employing an orthotopic lung adenocarcinoma model, the present study provides clinically relevant evidence that exercise-induced anti-inflammatory effects are preserved within lung-specific tumor environments.

Macroscopic and histological analyses confirmed comparable tumor presence in exercised and non-exercised cachexia mice, indicating that endurance exercise did not exert direct antitumor effects. This distinction is particularly important in the context of oncological supportive care, where exercise interventions are expected to improve host resilience rather than suppress tumor growth.[11,20]

Although endurance training significantly reduced TNF-α levels, it did not fully prevent skeletal muscle loss. Cachexia-associated muscle wasting involves multiple mechanisms beyond inflammation, including activation of proteolytic pathways and impaired anabolic signaling.[9,21,22] These findings suggest that inflammation control alone may be insufficient to preserve muscle mass and that combined therapeutic strategies may be required.

Endurance exercise is known to induce systemic anti-inflammatory responses through myokine secretion, mitochondrial adaptation, and immune modulation.[12-14] Exercise-induced myokines may suppress pro-inflammatory cytokine production and contribute to systemic metabolic homeostasis, consistent with the significant main effect of exercise on TNF-α observed in this study. In particular, exercise-induced interleukin-6, which is transiently elevated, may suppress TNF-α production and contribute to anti-inflammatory regulation.[23-27] These mechanisms likely explain the observed reduction in systemic inflammation.

In the context of cancer cachexia, systemic inflammation represents a critical upstream driver of metabolic dysregulation and muscle catabolism.[8-10] Experimental evidence suggests that sustained TNF-α signaling activates proteolytic pathways, including the ubiquitin–proteasome system and autophagy, while simultaneously impairing anabolic signaling through insulin-like growth factor-1 and mammalian target of rapamycin pathways.[21,28] Although the present study did not directly assess these downstream pathways, the observed reduction in circulating TNF-α provides indirect evidence that endurance exercise may mitigate key inflammatory triggers involved in cachexia progression.

Importantly, the present findings should be interpreted within the framework of supportive oncology rather than antitumor intervention. Macroscopic and histological analyses demonstrated comparable tumor presence in exercised and non-exercised cachexia mice, indicating that

endurance training did not inhibit tumor establishment or growth. This distinction reinforces the concept that exercise primarily exerts host-directed benefits, targeting systemic inflammation and metabolic stability rather than directly affecting tumor burden.[29] Such a distinction is essential in oncology, where non-pharmacological interventions are often evaluated for their capacity to improve tolerance to treatment and overall functional status rather than tumor control.

The use of an orthotopic lung adenocarcinoma model further strengthens the translational relevance of the present study. Subcutaneous tumor models, although technically simple, fail to replicate lung-specific tumor–host interactions, including regional inflammation, hypoxia, and respiratory burden.[17,30] By contrast, orthotopic implantation allows tumor growth within the lung parenchyma, reproducing both local pathological features and systemic consequences relevant to lung cancer–associated cachexia. The confirmation of tumor presence by both macroscopic inspection and histological evaluation supports the validity of this model for studying supportive interventions.

Despite the anti-inflammatory effects observed, endurance training did not result in statistically significant preservation of gastrocnemius muscle mass in tumor-bearing mice. This finding underscores the multifactorial nature of cachexia and highlights the limitations of targeting inflammation alone. Skeletal muscle wasting in cancer cachexia is driven not only by inflammatory cytokines but also by reduced physical activity, altered energy balance, and tumor-derived catabolic factors.[9,21,31] Therefore, exercise-induced reductions in TNF-α may represent an important but insufficient component of a comprehensive cachexia intervention.

From a translational perspective, these findings suggest that endurance exercise may serve as a foundational element within multimodal cachexia management strategies. Combination approaches integrating exercise with nutritional supplementation, anabolic agents, or anti-inflammatory pharmacotherapy may be required to achieve meaningful preservation of muscle mass and functional capacity.[11,32] The present pilot data provide a biological rationale for such integrative strategies by demonstrating that exercise can modulate systemic inflammation even in the presence of established lung tumors.

Furthermore, the exploratory nature of this study should be emphasized. As a pilot investigation, the primary objective was to evaluate feasibility and generate mechanistic hypotheses rather than to establish definitive therapeutic efficacy. The observed effect sizes for TNF-α modulation suggest biologically relevant trends that warrant confirmation in larger, adequately powered studies incorporating additional inflammatory markers and functional outcomes.

The clinical implications of these findings are noteworthy. Pulmonary rehabilitation and exercise therapy are increasingly incorporated into supportive care programs for patients with lung cancer.[33,34] While exercise alone may not reverse established cachexia, its capacity to attenuate systemic inflammation may improve physical function, treatment tolerance, and quality of life.

Beyond the reduction of TNF-α levels observed in the present study, it is important to consider the broader physiological implications of exercise-induced modulation of systemic inflammation in cancer cachexia. Chronic inflammation in cachexia is not only a consequence of tumor burden but also a driver of metabolic dysregulation affecting multiple organ systems, including skeletal muscle, adipose tissue, and the liver. In this context, endurance exercise may function as a systemic regulator that partially restores homeostatic balance disrupted by tumor-induced inflammatory signaling.

Emerging evidence suggests that exercise-induced anti-inflammatory effects extend beyond the suppression of individual cytokines and involve coordinated regulation of immune cell function. For example, endurance exercise has been shown to influence macrophage polarization, shifting the balance from pro-inflammatory M1 phenotypes toward anti-inflammatory M2 phenotypes. This shift may contribute to the attenuation of systemic inflammation and tissue catabolism. Although immune cell profiles were not assessed in the present study, the observed reduction in TNF-α may reflect broader immunomodulatory adaptations induced by repeated endurance exercise.

In addition to immune modulation, exercise may influence energy metabolism, which is profoundly altered in cancer cachexia. Tumor-bearing hosts often exhibit increased resting energy expenditure and impaired substrate utilization, leading to progressive weight loss and muscle wasting. Endurance training is known to enhance mitochondrial function, increase oxidative capacity, and improve metabolic flexibility in skeletal muscle. These adaptations may counteract some of the metabolic disturbances associated with cachexia, even without complete preservation of muscle mass. Therefore, the reduction in TNF-α observed in this study may represent one aspect of a more comprehensive metabolic adaptation induced by exercise.

Another important consideration is the interaction between systemic inflammation and the neuroendocrine system. Cancer cachexia is associated with dysregulation

of hormonal pathways, including increased glucocorticoid activity and altered insulin signaling. Exercise has been reported to modulate these pathways by improving insulin sensitivity and reducing stress hormone levels. Although these parameters were not directly measured in the present study, it is plausible that endurance training contributed to a more favorable neuroendocrine environment, which in turn may have influenced inflammatory signaling and energy balance.

Furthermore, the temporal dynamics of exercise-induced adaptations should be considered. The present study evaluated outcomes at a single endpoint; however, the anti-inflammatory effects of exercise may evolve over time. Acute exercise induces transient increases in certain cytokines, followed by longer-term reductions in basal inflammatory levels with repeated training. Longitudinal assessment of inflammatory markers would provide further insight into the time course of these adaptations and their relationship with disease progression.

From a mechanistic standpoint, it is also important to recognize that TNF-α interacts with multiple downstream signaling pathways that regulate muscle protein turnover. Activation of nuclear factor-kappa B signaling and upregulation of muscle-specific ubiquitin ligases contribute to protein degradation in cachexia. Although the present study did not directly examine these molecular pathways, the observed decrease in TNF-α suggests the potential for downstream suppression of catabolic signaling. Future studies incorporating molecular analyses of muscle tissue would be valuable in elucidating these mechanisms.

The absence of a significant effect of exercise on tumor burden in this study should not be interpreted as a limitation of exercise per se but rather as an expected finding within the framework of supportive care. In clinical oncology, exercise interventions are not primarily intended to reduce tumor size but to improve host resilience and functional capacity. The present findings align with this paradigm, demonstrating that exercise can modulate systemic inflammation without directly influencing tumor growth. This distinction is essential for appropriately positioning exercise as a complementary strategy in cancer management.

Importantly, the concept of "stabilization" rather than "reversal" may be particularly relevant in the context of advanced disease. Cancer cachexia is often progressive and difficult to reverse; therefore, interventions that slow or stabilize the trajectory of decline may have meaningful clinical benefits. The attenuation of TNF-α observed in this study suggests that endurance exercise may help maintain physiological equilibrium, even if complete recovery of muscle mass is not achieved. This perspective aligns with clinical observations that exercise can improve quality of life and physical function in patients with advanced cancer.

From a translational perspective, these findings support integrating structured endurance exercise into multidisciplinary cachexia management programs. However, the optimal exercise prescription, including intensity, duration, and timing relative to disease stage, remains to be determined. It is possible that different exercise modalities or combined training approaches may yield greater benefits for muscle preservation while maintaining anti-inflammatory effects. Future research should explore these variables to optimize intervention strategies.

Finally, the present findings contribute to a growing body of evidence supporting the role of exercise as a biologically active intervention in oncology. Rather than being viewed solely as supportive care, exercise may be considered a form of "metabolic therapy" that targets systemic dysregulation associated with cancer and its complications. By modulating inflammatory pathways, metabolic function, and potentially immune responses, endurance exercise represents a promising adjunctive approach to managing cancer cachexia.

Several limitations of the present study should be acknowledged. First, this investigation was designed as an expanded pilot study, and the sample size in each experimental group was relatively small. Second, only a single inflammatory marker, TNF-α, was evaluated in the present study. Cancer cachexia is mediated by a complex network of cytokines and signaling pathways, including interleukin-6, interleukin-1β, and transforming growth factor-β, as well as downstream regulators of proteolysis and mitochondrial function. The exclusive focus on TNF-α limits the ability to fully characterize the inflammatory milieu associated with lung adenocarcinoma–induced cachexia. Future studies incorporating a broader panel of inflammatory and metabolic biomarkers are warranted. Third, although endurance training significantly attenuated TNF-α elevation, it did not result in a statistically significant preservation of gastrocnemius muscle mass in tumor-bearing mice. This finding suggests that suppression of systemic inflammation alone may be insufficient to counteract cachexia-associated muscle wasting. Fourth, immunodeficient nude mice were used to establish the orthotopic lung adenocarcinoma model. While this approach enables stable tumor engraftment, it precludes evaluation of adaptive immune responses and immune–exercise interactions. Given the growing recognition of immune modulation as a key mechanism underlying exercise-induced benefits in cancer, future studies using immunocompetent models are needed to

enhance translational relevance. Fifth, tumor burden was not quantitatively assessed in this study. Although macroscopic and histological analyses confirmed tumor presence in both cachexia groups, detailed quantitative comparisons, such as the number of tumor nodules or tumor size, were not performed. Therefore, the potential effects of endurance training on tumor progression cannot be fully determined. Future studies incorporating quantitative tumor burden assessments are warranted to further clarify the relationship between exercise and tumor progression. Sixth, the gross appearance images of mice (**Figure 1**) were not obtained from perfectly standardized angles or positions. Although the images qualitatively demonstrate differences in body condition between groups, slight variations in image acquisition may limit direct visual comparison. Future studies should incorporate standardized imaging protocols to improve comparability. Finally, functional outcomes such as exercise capacity, muscle strength, or behavioral activity were not assessed. Although biochemical and morphological indices provide important mechanistic insights, functional measurements are essential to determine the clinical significance of exercise interventions in cancer cachexia. Incorporation of functional endpoints will be critical in future investigations.

5. Conclusion

In conclusion, the present expanded pilot study demonstrates that endurance training significantly attenuates systemic inflammation, as reflected by reduced circulating TNF-α levels, in a murine model of lung adenocarcinoma–associated cachexia. Importantly, this anti-inflammatory effect was observed without suppression of tumor establishment, as confirmed by macroscopic and histological lung findings, indicating that endurance exercise primarily exerts host-directed rather than antitumor effects.

The findings highlight the potential role of endurance exercise as a supportive intervention targeting inflammation in cancer cachexia. Although endurance training did not fully prevent skeletal muscle loss in tumor-bearing mice, the observed modulation of TNF-α suggests that exercise may help stabilize the systemic metabolic environment underlying cachexia progression. From a translational perspective, these results support integrating endurance-based exercise programs into multimodal supportive care strategies for patients with lung cancer.

Moreover, the use of an orthotopic lung adenocarcinoma model enhances the clinical relevance of the present findings by recapitulating lung-specific tumor–host interactions and systemic consequences. This model provides a valuable platform for future investigations aimed at optimizing exercise prescriptions and exploring combinatorial approaches that integrate exercise with nutritional or pharmacological therapies.

Taken together, the present study provides preclinical evidence that endurance exercise exerts biologically meaningful anti-inflammatory effects in lung adenocarcinoma–associated cachexia. These findings underscore the importance of supportive, non-pharmacological interventions in oncology and warrant further investigation in larger, mechanistically oriented preclinical studies and, ultimately, in clinical trials targeting cancer cachexia.

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Conflict of interest

The authors declare no conflicts of interest associated with this study.

Author contributions

Conceptualization: Naoki Maki, Takahiro Yanagihara, Kazuto Sugai, Tomoyuki Kawamura, Yukio Sato

Formal analysis: Naoki Maki, Harumi Sakamoto, Keisuke Taniguchi

Investigation: Naoki Maki

Methodology: Naoki Maki, Takahiro Yanagihara, Kazuto Sugai, Tomoyuki Kawamura,
Yusuke Saeki, Naohiro Kobayashi, Hideo Ichimura, Yukio Sato

Writing–original draft: Naoki Maki

Writing–review & editing: Naoki Maki, Yanagihara, Kazuto Sugai, Tomoyuki Kawamura, Yusuke Saeki, Naohiro Kobayashi, Hideo Ichimura, Yukio Sato

Ethics approval and consent to participate

All experimental procedures were approved by the Animal Experiment Committee of the University of Tsukuba (approval number: 21-461).

Consent for publication

Not applicable.

Availability of data

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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