ORIGINAL ARTICLE

High-intensity interval training slows down tumor progression in mice bearing Lewis lung carcinoma

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Abstract

Background We aimed to determine whether a short-term high-intensity interval training (HIIT) protocol could counteract tumor progression in an experimental model of lung cancer.

Methods Mice were injected subcutaneously with Lewis Lung Carcinoma (LLC) cells and then randomly assigned into two groups: sedentary mice (LLC group) or mice submitted to HIIT (LLC + HIIT group).

Results LLC + HIIT group had lower tumor mass than LLC group (-52% after 18 days), with no differences in glycolytic activity as measured by PET/CT imaging. HIIT increased Cd274 (PD-L1) mRNA expression by ~6 folds and Vegfa mRNA expression by 2.5 folds, suggesting that HIIT stimulates local inflammation and angiogenesis in LLC tumors. Additionally, HIIT improved running capacity, skeletal muscle contractility and survival rate in LLC tumor-bearing mice.

Conclusions These novel findings demonstrate that a short-term HIIT protocol slows down tumor progression, ultimately increasing survival in LLC tumor-bearing mice. Thus, this study provides novel pre-clinical evidence that exercise training may be a beneficial co-therapy for lung cancer.

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Introduction

Endurance capacity predicts early mortality and the enrollment in regular physical activity reduces the incidence of several diseases [1, 2]. Exercise training induces pleiotropic effects in the body, regulating the rate of energy production, blood flow, and substrate utilization [3]. Over the past decades, aerobic exercise training has been applied not only as a preventive strategy, but also as a non-pharmacological therapy or co-adjuvant treatment for cardiovascular, metabolic/endocrine, rheumatic and neurological diseases [4–9]. In the oncology field, more than 200 original studies have now analyzed the role of aerobic exercise training (or at least regular physical activity) in cancer patients [10]. Comprehensive reviews of those studies have proposed that a structured exercise training protocol is a safe and effective co-therapy for most of cancer patients during and after the first-line treatment [10].

Due to the advances in cancer treatment, cancer-specific survival rates for the 10 most common malignancies improved dramatically in the last three decades and patients diagnosed with those malignancies now have sufficient longevity, except for lung cancer cases [10, 11]. Lung cancer is the leading cause of cancer-related deaths worldwide [12]. Most of lung cancer patients receive chemotherapy with or without radiation. Targeted drugs, such as inhibitors for epidermal growth factor receptor and anaplastic lymphoma kinase are also important for treatment of non-small cell lung cancer patients [11]. Despite pharmacological advances, adjuvant therapy and palliative supportive care for lung cancer patients is still limited. There is a paucity of well-designed studies to support the efficacy and safety of exercise training for lung cancer patients. The lack of studies can be partially explained by the frailty and

excessive fatigue presented by this population. In fact, most of advanced lung cancer patients display functional impairment [13, 14], which limits conventional physical exercise interventions.

Animal studies have been useful to assess the effects of aerobic exercise training in catabolic conditions. Pre-clinical studies also help to explore mechanisms underlying exercise benefits and to identify potential side effects. Remarkably, aerobic exercise training associated or not with pharmacological approaches can slow down tumor growth and attenuate cancer-induced muscle wasting in different animal models, such as the Colon-26 tumor-bearing mice [15, 16], B16F10 melanoma tumor-bearing mice [17], Walker 256 breast tumor-bearing rats [18, 19] and Lewis Lung Carcinoma (LLC) tumor-bearing mice [20]. While these studies demonstrated benefits with exercise training starting prior to tumor cells injection, exercise has limited effects when the protocol starts after tumor cells injection [17, 21].

To optimize the effects of aerobic exercise training, studies have tested and verified similar or even superior benefits of short-term high-intensity interval training (HIIT) protocols when compared to moderate-intensity aerobic exercise training in patients with cardiovascular or metabolic diseases [22–26]. For instance, a submaximal HIIT protocol consisting of four 4-minute bouts at 90% of maximal heart rate interspersed by 3-minute active recovery at 70% of maximal heart rate was superior than moderate-intensity continuous aerobic exercise training (70% of maximal heart rate) in reducing blood glucose level, improving endothelial function and enhancing muscle biogenesis in metabolic syndrome patients [26]. Accordingly, our group has demonstrated that a similar HIIT protocol was superior than moderate-intensity continuous aerobic training in improving aerobic capacity of myocardial infarcted rats [22]. The rationale behind HIIT protocol is that the interval design enable periods at higher intensities than could be possible during continuous exercise protocols [23, 26]. To our knowledge, there is no studies evaluating the effects of HIIT in cancer animal models.

We hypothesized that HIIT would slow down tumor progression in an experimental model of lung cancer. To test this hypothesis, we evaluate the effects of a short-term HIIT protocol in a severe murine cancer model. Our findings demonstrate that a short-term HIIT protocol attenuates tumor growth and improves endurance capacity in LLC tumor-bearing mice, indicating that HIIT might be considered a potential therapy to attenuate cancer progression and cancer-related fatigue.

Methods

Animal Model, Experimental Design and Ethics

Sixteen-week-old male C57BL/6 mice were used in the current study. Animals were housed in an animal facility under controlled temperature (21°C) with 12:12 hours light:dark cycle and had *ad libitum* access to standard laboratory chow and water. All mice were injected subcutaneously in the right flank with 10^6 LLC cells diluted in 100 µL of serum-free DMEM medium. LLC cell line was donated by Prof. Seelaender group from Institute of Biomedical Sciences of University of Sao Paulo (Sao Paulo, Brazil) and was cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, NY, US) medium supplemented with 10% fetal bovine serum before being injected into mice (Gibco, NY, US). One day after tumor cells injection, mice were randomly assigned into two experimental groups: 1) sedentary mice (LLC group) or 2) mice submitted to HIIT (LLC + HIIT group). For ethical purposes, mice were sacrificed whether they appeared moribund, indicating low probability of surviving for greater than 24 hours. Mice were killed by cervical dislocation under isoflurane anesthesia at 18 days post LLC cells injection. Tumors and *extensor digitorum longus* (EDL) muscles were carefully harvested and weighted. Carcass mass delta changes were calculated for each mouse after removing tumor mass from total body mass. Tumors were cut in two pieces and stored in −80 °C for further RNA extraction or fixed in 10% formalin for further histological analysis. EDL muscle was immediately used in *ex vivo* skeletal muscle experiments. In independent cohorts, 1) positron emission tomography/computed tomography (PET/CT) imaging was performed to evaluate ^{18}F-FDG uptake in the tumors, an indicator of tumor metabolic activity, and 2) running capacity was analyzed including additional "healthy" experimental groups to determine the effects of HIIT in the presence or absence of tumors. Sample size used for each experiment is indicated at figure legends.

This study was approved by the Ethical Committee of School of Physical Education and Sport, University of Sao Paulo (protocol 2012/01). All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, USA), and with ethical principles in animal research adopted by the Brazilian Council for the Control of Animal Experimentation.

High-Intensity Interval Training (HIIT) Protocol and Maximal Incremental Running Test

All mice were adapted to the treadmill running for five consecutive days. Adaptation consisted of 10 minutes running at low to moderate speed (<15 $m.min^{1}$) in a graded treadmill at 15° inclination. After the adaptation protocol, mice were injected with LLC cells. HIIT protocol started one day after tumor cells injection and comprised of exercise daily sessions. Each session consisted of 5 intervals of 3 minutes running at 18 $m.min^{-1}$ followed by 4 minutes running at 25 $m.min^{-1}$. HIIT protocol started one day after tumor cells injection and were carried on daily until 16 days post tumor cells injection. Mice were tested or sacrificed forty-eight hours after the last HIIT session. To test the efficacy of this short-term HIIT protocol, mice were submitted to a maximal incremental running test at 18 days post tumor cells injection. This test consisted of running on a graded treadmill at 15° inclination until exhaustion. The speed started at 6 $m.min^{-1}$ and was increased by 3 $m.min^{-1}$ every 3 min until mice were unable to run. Maximal speed achieved by each mouse and the time to exhaustion were recorded.

Histological Analysis

Tumors fixed in 10% formalin were embedded in paraffin, sectioned into 5 μm-thick sections, and stained with hematoxylin and eosin (HE) for microscopic observation at 400X magnification (Leica Qwin, Leica Microsystems, Germany). Twenty different images were acquired for each sample. To evaluate potential changes in tumor histopathological features, areas with close nuclei and areas with distant and/or pleomorphic nuclei were distinguished and quantified by a researcher (RFB) blinded to mice´s IDs. Areas of clustered nuclei represented the tumor parenchyma (high density of tumor cells), while areas with lower density of cells represented areas richer in tumor stroma. In addition, a pathologist performed qualitative analysis as previously described [27] . There were no clear differences between experimental groups. This information was added in the manuscript. Representative images of HE stains are presented.

Positron Emission Tomography (PET) and Computed Tomography (CT) imaging

Mice were imaged in a small-animal PET/SPECT/CT scanner (Triumph Trimodality, Gamma Medica-Ideas Inc., California, US). Each mouse was fasted for 4 to 5 hours, anesthetized with 3% isoflurane and injected into the penile vein with 18-30 MBq of ^{18}F-FDG. Mice were positioned in the scanner 45 min after tracer administration with the tumor located in the center of the field of view and a PET scan was acquired for 30min. CT images were acquired immediately after PET scan to identify the tumor location and body anatomy. Images were iteratively reconstructed using the algorithm OSEM-3D with 20 iterations and 4 subsets. Each image fusion and quantification were performed by using the PMOD software version 3.310 (PMOD Technologies LLC; Switzerland). A region of interest (ROI) was drawn for each tumor. Analysis were performed by an investigator (CGC) blinded to image´s IDs and the data corrected for basal blood glucose levels of each mouse (measured before tracer injection).

mRNA levels

RNA was isolated from frozen tumor samples using TRIzol reagent (Thermo Fisher Scientific, USA) and reverse transcribed using High Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, USA) accordantly to the manufacturer protocols. Before cDNA synthesis, RNA purity and concentration were determined spectrophotometrically by NanoDrop 2000 (Thermo Fisher Scientific, USA) and RNA integrity was checked by 1% agarose gel stained with Nancy-520 (Sigma-Aldrich, USA). cDNA was analyzed by quantitative real-time PCR (RT-qPCR). Reactions were performed using 12.5 ng of cDNA and 200 nmol of each primer mixed with SYBR Green PCR Master Mix (Thermo Fisher Scientific, US). Relative programmed death-ligand 1 (Cd274; PD-L1), fibroblast growth factor-inducible 14 (Fn14), tumor necrosis factor α (Tnfa), interleukin 6 (Il6), vascular endothelial growth factor A (Vegfa), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (Ppargc1a) mRNA levels were calculated using comparative CT method. TATA-binding protein (Tbp) was used as a housekeeping. Primer sequences are provided in Table 1.

Table 1. List of primer sequences used for RT-qPCR.

Gene	Forward (5' > 3')	Reverse (5' > 3')
Cd274 (PD-L1)	TCACTTGCTACGGGCGTTTAC	TGACGTTGCTGCCATACTCC
Fn14	CTGGTTTTGGCGCTGGTTTC	AGTCTCCTCTATGGGGGTAGTAAA
Tnfa	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Vegfa	GCACATAGAGAGAATGAGCTTCC	CTCCGCTCTGAACAAGGCT
Ppargc1a	TGATGTGAATGACTTGGATACAGACA	GCTCATTGTTGTACTGGTTGGATATG
Tbp	AAGAGAGCCACGGACAACTG	GCTAGTCTGGATTGTTCTTCACTCT

Ex vivo skeletal muscle contractility

Contraction capacity was analyzed in *extensor digitorum longus* (EDL) muscle. EDL was carefully harvested and immediately used in *ex vivo* experiments. Muscles were placed in a tissue bath contain aerated Krebs Ringer´s buffer (137mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM KH_2PO_4, 1mM $MgSO_4$, 24mM $NaHCO_3$, 11mM $C_6H_{12}O_6$; 22ºC; pH 7.4; 95% O_2 and 5% CO_2) and platinum-based electrodes. The proximal tendon of each muscle was fixed in a force transducer and equilibrated with Krebs Ringer´s buffer for 10 minutes. To identify the optimal muscle length, single electrical pulses (1Hz, 80V) were applied interspersed by two-minute rest intervals. EDL lengths were adjusted between each stimulus and the force output was recorded. After identifying the optimal muscle length (L_o), EDL rested in the Krebs Ringer´s buffer for 10 minutes. A force-frequency protocol was then conducted. Stimuli (0.2ms, 80V, with 1200ms and 350ms trains for *soleus* and EDL, respectively) were applied with successive frequency increases (*i.e.* 10, 20, 30, 40, 50, 80, 100 and 150 Hz). Each stimulus was interspersed by three-minute rest intervals. Force output was recorded, and data was presented as absolute force production or force production normalized by muscle mass.

Statistical analysis

Values are presented as mean ± standard error (SE) or individual values. Analyses were conducted using Graph Pad Prism 6 (Graph Pad Software Inc., USA). Unpaired Student *t* test was used to test differences between LLC and LLC + HIIT groups. Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was applied for *ex vivo* skeletal muscle contractility and running capacity experiments. Log-rank test was performed to compare survival rate. Statistical significance was set at $p < 0.05$.

Results

HIIT attenuates tumor growth and regulates genes involved in inflammation and angiogenesis in Lewis lung carcinoma tumor-bearing mice

To determine whether a short-term HIIT protocol would affect tumor growth in a severe murine cancer model, LLC tumors were harvested and weighted. LLC + HIIT group displayed 52% lower tumor mass than LLC group (**Figure 1a**), with no differences in areas richer in tumor stroma (**Figure 1b,c**). In addition, qualitative analysis did not demonstrate clear differences in tumor histology between experimental groups. Thus, these initial findings demonstrated that HIIT reduced tumor mass in LLC tumor-bearing mice, but did not affect tumor histopathological features, such as parenchyma to stroma ratio within tumors.

To explore whether HIIT could regulate tumor microenvironment, we evaluated levels of mRNA for some genes involved in inflammation (Cd274, Fn14, Tnfa and Il6) and angiogenesis (Vegfa and Ppargc1a) pathways. PD-L1 (Cd274) is a transmembrane protein that has been established as one of the main suppressors of immune response. This immunomodulatory role is attributed to the binding of PD-L1 to its receptor on the surface of different immune cells, resulting in an intracellular inhibitory signaling cascade that impairs tumor-infiltrating T cells. Notably, HIIT increased Cd274 mRNA levels by ~6 folds in LLC tumors (**Figure 1d**). No significant differences between experimental groups were observed for Tnfa and Il6, two well-known cytokines involved in local and systemic inflammation, and Fn14, a tumor necrosis factor receptor that contributes to carcinogenesis (**Figure 1d**). Additionally,

HIIT increased Vegfa mRNA expression by 2.5 folds, although no significant difference was observed in Ppargc1a mRNA expression (**Figure 1d**). Interestingly, we also found a positive correlation between Vegfa and Ppargc1a mRNA levels among all samples (r = 0.85). Vegfa and Ppargc1a encode proteins (VEGF A and PGC-1α, respectively) characterized as mediators of angiogenesis, which suggests that HIIT might stimulate angiogenesis in LLC tumors.

Cancer cells reprogram their energy metabolism, using glycolytic metabolism and exhibiting glucose preference even in the presence of oxygen. Thus, ^{18}F-FDG (an analogue of glucose) PET is a powerful imaging tool for the detection of different tumors types, including non-small cell lung cancer in humans. Here, we evaluated glycolytic activity in LLC tumors by using ^{18}F-FDG PET/CT scanning. However, no differences were observed in the ^{18}F-FDG uptake between LLC and LLC + HIIT groups (**Figure 1e,f**). Together, these data indicate that HIIT increased mRNA levels of genes involved in inflammation and angiogenesis processes but did not affect the overall glucose uptake in LLC tumors.

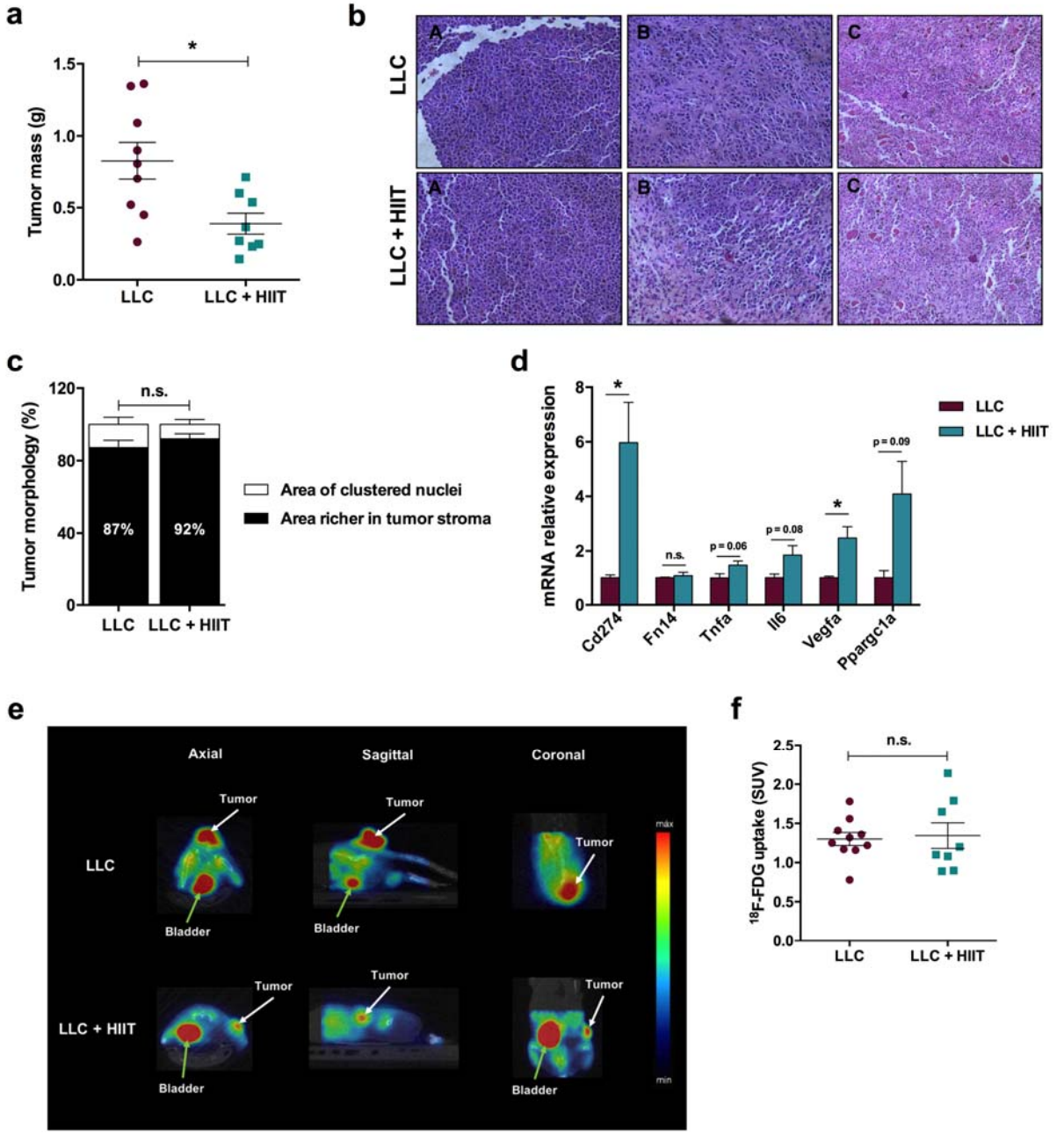
HIIT improves skeletal muscle contractility, running capacity, and survival in Lewis lung carcinoma tumor-bearing mice

Cachexia syndrome remains as a key problem for cancer patients, and LLC tumor-bearing mice is a standardized cancer cachexia murine model. HIIT protocol did not affect body mass (**Figure 2a**), but significantly improved skeletal muscle contractility as evaluated by the twitch tension in isolated EDL muscle (**Figure 2b,c**). Importantly, this beneficial effect in the muscle contractility was not due to difference in muscle mass (**Figure 2d**).

We next determined whether a short-term HIIT protocol would improve endurance capacity in this severe murine cancer model. Preliminary data demonstrated that LLC + HIIT group displayed longer time to exhaustion than sedentary LLC group (data not shown), suggesting that the HIIT protocol is effective to improve endurance capacity in LLC tumor-bearing mice. Because this study was primarily designed to investigate the effects of HIIT on tumor growth, we did not include additional healthy control groups in the experiments presented above. However, we performed an additional experiment including two heathy groups to understand the effects of HIIT in the presence or absence of tumors. Interestingly, short-term HIIT protocol did not affect running capacity in healthy control mice, but clearly normalized running capacity in LLC tumor-bearing mice at 18 days post tumor cells injection (**Figure 2e**). Remarkably, in this independent mouse cohort, LLC + HIIT group also presented smaller tumors than LLC group (**Figure 2f**).

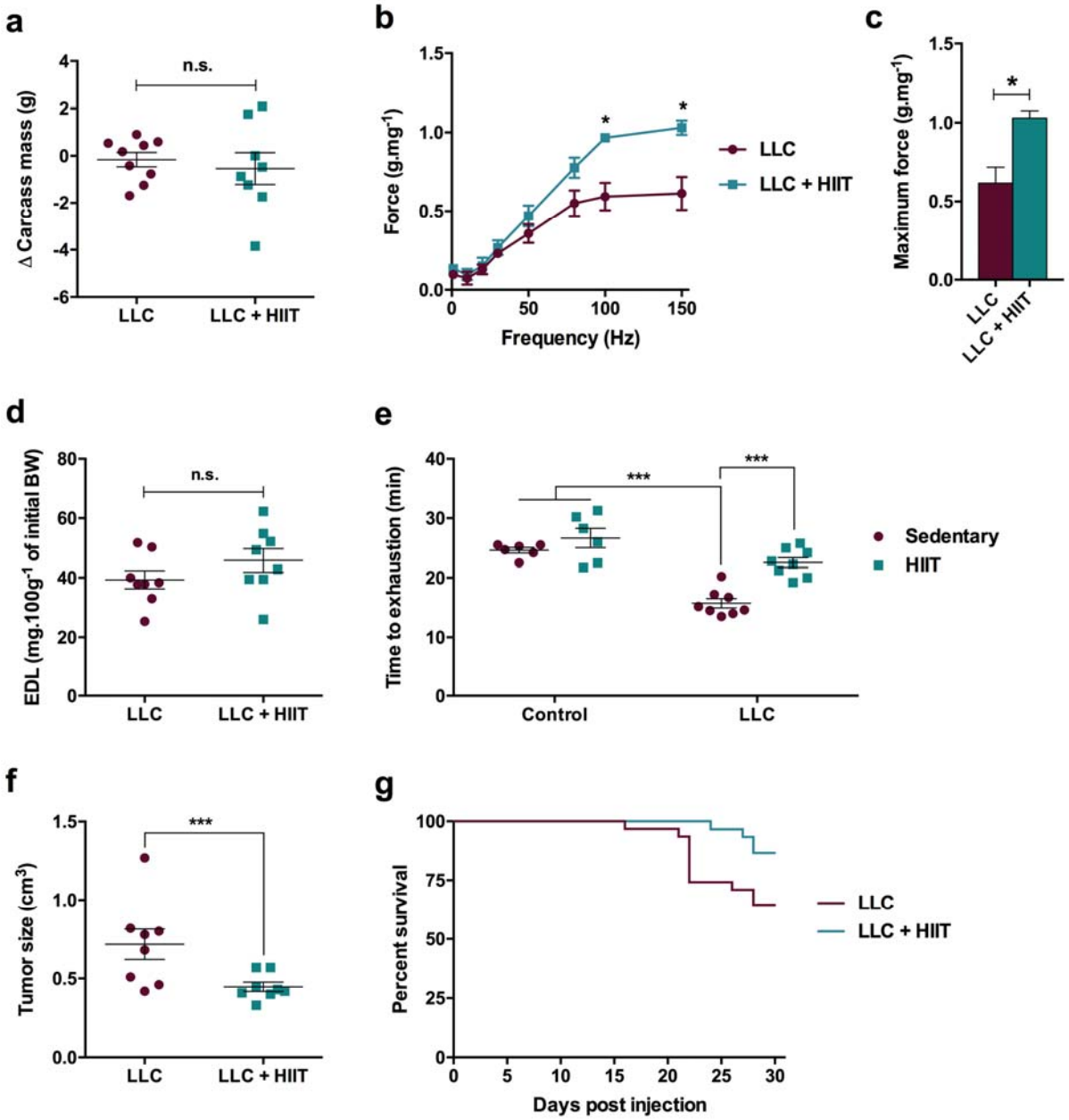
We compared survival rate between LLC and LLC + HIIT groups for 30 days. LLC + HIIT group presented a significant lower mortality rate than LLC group (**Figure 2g**). Altogether, these novel findings demonstrated that a short-term HIIT protocol improves skeletal muscle contractility, endurance capacity and survival in LLC tumor-bearing mice.

Figure 1.



Short term high-intensity interval training (HIIT) attenuates tumor growth in Lewis lung carcinoma tumor-bearing mice. (**a**) Tumor mass. n = 8-9. (**b,c**) Tumor histopathological features assessed by histological analysis after tumors were stained with hematoxylin and eosin. n = 8-9. (**d**) mRNA expression for genes involved in inflammation and angiogenesis pathways. n = 5-7. (**e,f**) Glucose metabolism in LLC tumors. ^{18}F-FDG Standardized Uptake Value (SUV) was corrected for blood glucose. n = 8-10. Data are presented as mean ± s.e.m with dots as individual values. *$p < 0.05$. n.s. indicates not significant differences.

Figure 2.



Short term high-intensity interval training (HIIT) improves running capacity in Lewis lung carcinoma tumor-bearing mice. (**a**) Body mass delta change. n = 8-9. (**b,c**) Skeletal muscle contraction capacity. n = 3. (**d**) *Extensor digitorum longus* (EDL) muscle mass. n = 8-9. (**e**) Time to exhaustion in a maximal incremental running test performed at 18 days post tumor cells injection. n = 6-8. (**f**) Tumor size. n = 8. (**g**) Survival rate. n = 30-31. Log-rank test revealed a *p* value of 0.03 between survival rates. Data are presented as mean ± s.e.m with dots as individual values. **p* < 0.05; ****p* < 0.001. n.s. indicates not significant differences.

Discussion

We provided pre-clinical evidence that HIIT is a potential non-pharmacological therapy for cancer. Our main finding indicates that HIIT reduced tumor mass by 52%, ultimately increasing survival in LLC tumor-bearing mice. Similarly, Pedersen et al (2016) reported that free wheel running reduced tumor mass by 56% in the same LLC model. Consistent with this finding, wheel running also attenuated tumor growth in B16F10 melanoma tumor-bearing mice and reduced tumor incidence in mice injected with diethylnitrosamine [17]. In the B16F10 melanoma model, authors reported that exercise benefits occurred when the wheel running protocol started weeks prior to tumor cells injection, but not when it started post tumor cells injection [17]. Instead, in the current study we found that a short-term HIIT protocol starting after tumor cells injection could reduce tumor mass. Moreover, Penna et al (2011) reported that a moderate-intensity aerobic exercise training protocol (five days/week treadmill running sessions at 14 m/min for 45 min) associated with administration of the omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) reduced tumor mass by only ˜20% in LLC tumor-bearing mice [20]. Thus, although a direct comparison cannot be made, the HIIT protocol seems more efficient than low to moderate-intensity continuous exercise training protocols in attenuating tumor growth. Further studies comparing HIIT *versus* continuous exercise training protocols in cancer models are still necessary to confirm (or not) this premise.

The reduction of tumor mass was associated with changes in mRNA levels of genes involved in inflammation and angiogenesis processes. HIIT clearly increased PD-L1 levels, a ligand of programmed cell death protein 1 (PD-1). PD-L1/PD-1 interaction transmits an inhibitory signal into T cell, which at last instance reduces cytokine production and suppresses T cell proliferation. Thus, PD-L1/PD-1 ensures that immune system is activated during an appropriate time, minimizing chronic inflammation [29]. However, even though the HIIT protocol has increased the Cd274 (PD-L1) mRNA levels in LLC tumors, it did not reduce pro-inflammatory cytokines production. Conversely, we observed a tendency to increase Tnfa and Il6 levels in the LLC + HIIT group. Therefore, we speculated that a compensatory PD-L1 activation is occurring during the repression of tumor growth, but it was not sufficient to suppress T cell proliferation. Supporting our findings, Pedersen et al (2016) observed higher levels of some pro-inflammatory cytokines, including TNF-α and IL-6 levels in LLC tumors after tumor-bearing mice were exposed to the wheel running protocol [17]. In that study, higher levels of pro-inflammatory cytokines were directly associated with immune system recruitment and the depletion of natural killers cells blunted the positive effects of exercise in the B16F10 melanoma mice model [17]. Regarding to angiogenesis markers, HIIT increased Vegfa mRNA levels, a mediator of angiogenesis [30–32]. Remarkably, Betof et al (2015) demonstrated that wheel running increased vessel perfusion and reduced hypoxia in mice orthotopically implanted with estrogen receptor–negative or positive breast tumor cells. Importantly, authors reported ~1.5 fold increase in Vegfa mRNA levels in the exercised group when compared to the control [33]. This is an important point since dysfunctional endothelium development is also a consequence of pro-angiogenic factors in tumors, including the VEGFA [34]. Taken these data together, we speculate that aerobic exercise training is a potential inductor of tumor perfusion, resulting in a more “normalized” tumor microenvironment. Despite the effects of HIIT in inflammatory and angiogenesis markers, we did not observe any HIIT effect in the LLC tumor glucose uptake capacity, suggesting that HIIT did not affect glycolytic metabolism in LLC tumor microenvironment.

Most of advanced oncologic patients display skeletal muscle wasting associated with functional impairment, and lung cancer patients with cachexia display shorter survival than non-cachectic ones [35]. In this context, the lack of therapies for cancer cachexia is still evident [36]. By using LLC tumor-bearing mice, Penna et al (2011) have previously demonstrated that moderate-intensity aerobic exercise training protocol associated with EPA administration mitigated muscle wasting [20]. In another study, Pin et al (2015) evaluated the effect of erythropoietin, the most effective pharmacological treatment for anemia, associated with the same exercise training protocol mentioned above and demonstrated that this combined approach did not attenuate loss of skeletal muscle mass, but promoted mitochondrial biogenesis and improved muscle function [21]. Likewise, even though we did not observe any effect in skeletal muscle mass, HIIT clearly increased muscle contraction capacity during *ex vivo* skeletal muscle experiments, which suggests that HIIT induces intrinsic adaptations in skeletal muscle contractile properties. This positive effect may be explained by 1) a direct HIIT-induced effect in the skeletal muscle metabolism or 2) an indirect effect, since

HIIT have attenuated tumor growth and the reduced tumor mass could produce lower levels of "cachectic factors". In summary, these findings indicate that aerobic exercise training may be considered a potential therapy to attenuate cancer related fatigue.

We acknowledge limitations in our study. First, we aimed to understand the effects of HIIT without any additional treatment during LLC tumor progression. However, this approach is far from translation to clinical practice since chemotherapy and other targeted drugs are necessary for treatment of lung cancer patients. Thus, further studies combining HIIT with pharmacological therapies are still necessary. Second, mice were injected subcutaneously with LLC. Further studies including orthotopic lung tumor models are recommend. Finally, additional analyses, such as cell proliferation, necrosis and apoptosis, are still needed to clarify the mechanisms underlying the beneficial effects of exercise training during tumor progression.

In summary, the present data demonstrated that a short-term HIIT protocol attenuated tumor growth in LLC tumor-bearing mice, and reduced tumor mass was associated with changes in mRNA levels of genes involved in inflammation and angiogenesis. Additionally, HIIT improved running capacity, skeletal muscle function and survival in LLC tumor-bearing mice. Hence, the present study provided a foundation for future work as well as pre-clinical evidence that HIIT may be considered a potential co-therapy for lung cancer.

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Authors' contributions

CRRA and PCB conceived and designed the study. PCB and RC provide all the laboratory support. CRRA, GCT, WN, NRA and CMM carried out the animal procedures. RFPB performed the histological analysis. CRRA, DPF, CGC and ATG performed the PET/CT experiments and analysis. CRRA and WN performed the RT-qPCR experiments and analysis. CRRA, RC and PCB performed data analysis and wrote the manuscript. All authors have participated in the manuscript review. All authors approved the final manuscript.

Conflicts of interest

None

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