Resistance exercise attenuates skeletal muscle oxidative stress, systemic pro-inflammatory state, and cachexia in Walker-256 tumor-bearing rats.

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Abstract

The aim of this study was to investigate the effects of resistance exercise training (RET) on oxidative stress, systemic inflammatory markers and muscle wasting in Walker-256 tumor-bearing rats. Male (Wistar) rats were divided into 4 groups: sedentary controls (n=9); tumor-bearing (n=9); exercised (n=9); and tumor-bearing exercised (n=10). Exercised and tumor-bearing exercised rats were exposed to resistance exercise of climbing a ladder apparatus with weights tied to their tails during 6-wks. The physical activity of control and tumor-bearing rats was confined to the space of the cage. After this period, tumor-bearing and tumor-bearing exercised animals were inoculated subcutaneously with Walker-256 tumor cells (11.0x10^7 cells in 0.5 ml of PBS) while control and exercised rats were injected with vehicle. Following inoculation, rats maintained resistance exercise training (exercised and tumor-bearing exercised) or sedentary behavior (control and tumor-bearing) for 12 more days, after which they were euthanized. Results showed muscle wasting in T animals, with body weight loss, increased systemic leukocytes and inflammatory interleukins as well as muscular oxidative stress and reduced mTOR signaling. In contrast, RET in tumor-bearing exercised group was able to mitigate the reduced body weight and muscle wasting with the attenuation of muscle oxidative stress and systemic inflammatory markers. RET also prevented loss of muscle strength associated with tumor development. RET however, did not prevent the muscle proteolysis signaling via FBXO32 gene mRNA expression in tumor-bearing group. In conclusion, RET performed prior tumor implantation prevents cachexia development by attenuating tumor-induced systemic pro-inflammatory condition with muscle oxidative stress and muscle damage.

Keywords: Strength training, oxidative damage, interleukins, muscle proteolysis, muscle wasting.
Introduction

Muscle wasting is the principal component of cancer cachexia, leading to progressive impairment of work capacity (Tan and Fearon 2008), which compromises the effectiveness of therapeutic treatment and constitutes a stronger hallmark of poor prognosis in cancer patients (Galvão et al. 2010). Several studies suggest that cancer cachexia results from the imbalance between degradation and protein synthesis, mediated among others by cytokines and reactive oxygen species (Balkwill and Mantovani 2012; Powers et al. 2016). The local enhanced oxidative stress and the up-regulation of pro-inflammatory cytokines may promote muscular catabolism via ubiquitin-proteasome system, especially by transcription factor nuclear factor-kB (NF-kB) (Argilés et al. 2014). This factor targets several muscular genes, such as the FBXO32, with the consequent enhanced activity of the ubiquitin-proteasome pathway (Gomes-Marcondes and Tisdale 2002). Thus, different therapeutic strategies have been tested to prevent or treat cancer-induced muscle wasting but none of them seem appropriate (Fearon et al. 2013).

Resistance exercise training (RET) is a well-known strategy to promote health and wellness (ACSM 2009), with potential favorable impacts on muscular phenotype. Indeed, the growing use of animal models of carcinogen-induced tumors in combination with exercise protocols has produced promising results regarding the potential benefits of RET in attenuating the effects caused by the tumor growth (Mallick et al. 2015). However, studies using models of carcinogens-induced tumors are generally not focused on skeletal muscle wasting as primary event (Bennani-Baiti and Walsh 2011; Donatto et al. 2013; Frajacomo et al. 2015). Moreover, although the use of a specific carcinogen tends to induce cancer predominantly in a defined organ, it is important to note that all those compounds have direct harmful consequences in different cell types,
and may motivate the occurrence of diverse types of cancer simultaneously in the same animal (Teixeira-Guedes et al. 2014). This situation compromises the homogeneity of the studied sample and, consequently, the obtained data and conclusions drawn.

Comparing with the carcinogen models, the use of solid tumor models to test the effect of RET protocols on muscle wasting has several advantages. The appropriate control of the amount of tumor cells injected in each animal, the homogeneity of tumor development, and the facility to monitor growth of the subcutaneous solid tumor along the experimental period of time (Todor et al. 2015) are the main examples. The Walker-256 tumor is a solid breast carcinosarcoma suitable to be used as an experimental model to induce muscle wasting in rats (Tayek et al. 1986). Nevertheless, independently of the cancer model, it must be noted that endurance training is the most studied type of training (Faustino-Rocha et al. 2013; Frajacomo et al. 2015; Padrão et al. 2015), which results in a scarcity of data elucidating the influence of RET on cancer-induced cachexia. Consequently, considering the lack of studies elucidating the influence of RET on cancer-induced muscle wasting and the disadvantages of using carcinogenic models for this purpose, the aim of this study was to investigate the effects of RET on the cachexia development in a solid tumor model, the Walker-256.

**Methods**

*Animals and experimental design.*

A total of thirty-seven male Wistar rats, weighing 252.4±19.4 g, were obtained from the Biological Sciences Center at the State University of Londrina. All procedures were approved by the Ethics Committee for Animal Use at the same institution, and were in accordance with the Guidelines of the Brazilian College of experiments with animals (COBEA). The rats were housed in collective cages on inverted 12-h-light/-dark cycle
at a mean temperature of 22°C, with free access to food and water throughout the experimental period. In addition, all groups were fed with the same standard diet (carbohydrates 62.7%, protein 24.8% and fat 12.4%). The body weight was recorded every 2 days. After one week of acclimatization, rats were randomly divided into four groups as follows: control (n=9), tumor-bearing group (n=9), resistance exercised group (n=9), and tumor-bearing resistance exercised group (n=10). Both exercised and tumor-bearing exercised groups were submitted to a progressive RET routine as described below. Rats from control and tumor-bearing remained sedentary during all experimental protocol, with physical activity restricted to their cage space. Six weeks after beginning of the RET, Walker-256 tumor cells were inoculated at the right flank of rats from tumor-bearing and tumor-bearing exercised, while animals from control and exercised received vehicle. Following inoculation, rats maintained RET (exercised and tumor-bearing exercised) or sedentary behavior (control and tumor-bearing) for 12 additional days, when they were then euthanized for tissue collection as described below. The experimental protocol is synthesized in Figure 1.

*Insert Figure 1 here*

**Maximal strength test**

The maximal strength test was performed according to previously described by (Hornberger and Farrar 2004). One week before starting RET protocol, all rats were familiarized with the apparatus. Initially, rats were placed at the lower part of the ladder and stimulated to climb by pushing the animal to initiate movements. Push stimuli were performed until each animal was capable of complete an entire climb. At the top of the ladder, rats were allowed to rest two minutes. This procedure was repeated for 5 consecutive days. At the end of the acclimatization week, rats were submitted to
voluntarily climb the ladder three consecutive times without stimulus. No additional load was used during this period. Immediately before beginning the experimental protocol (at day 0) a maximal training load test was carried out to determine animals’ maximal strength. Initially, all rats started the climbing test with 75% of its body weight attached to their tail. After climbing successfully with the initial load, an additional 30g was added to be lifted. This procedure was successively repeated until achieving a load that unable the animal to climb the complete ladder length during three consecutive attempts. In those cases, the weight load pulled in the anterior successful climbing attempt defined as the animal’s maximal strength. This test was repeated for all groups at days 28 and 52 of the experimental protocol.

**Resistance exercise training**

RET consisted of a ladder climbing (1.1 x 0.18 m, 2-cm grid, 90° incline) protocol that was adapted from (Hornberger and Farrar 2004). The length of the ladder facilitated 8-12 dynamic movements by the animals per climb. At the top of the ladder, a dark covered chamber was constructed (20 x 20 x 20 cm) for interval resting between climbing bouts. Rats from exercised and tumor-bearing exercised were submitted to a progressive RET with sessions consisted of 4 to 8 ladder climbs carrying 50%, 75%, 90%, and 100% of their maximal carrying capacity achieved in the previous training session. The RET was conducted three times per week over 52 days, for a total of 25 training sessions. With the exception of days 28 and 52 of maximal strength test performance, the physical activity of rats from control and tumor-bearing during the remained time period of experimental protocol was confined to the space of the cage.

**Tumor implantation**
Tumor cells were obtained from ascetic intraperitoneal tumor (2.0x10⁶ cells in 0.5 ml PBS) in host animals. At the moment of inoculation, tumor cells were removed from the peritoneal cavity of host rats (with 8 µl/ml of 5000 IU/ml heparin) and centrifuged at 1000 x g for the intermediate phase collection. The percentage of viable cells was determined by trypan blue dye exclusion method (nonviable cells stained blue), using a Neubauer chamber. Tumor-bearing and tumor-bearing exercised groups received a Walker-256 cell suspension (11.0x10⁷ cells in 0.5 ml of PBS) injected subcutaneously into the right flank of the animals and animals were accompanied for 12 days. Control and exercised animals were inoculated in the same region with 0.5-ml of PBS.

Necropsy and tissue preparation

Forty-eight hours after the last training session (at 54 days), rats were weighed and anesthetized with an intramuscular injection of a ketamine and xylazine mixture (65 mg/kg) between 8 and 12 a.m. Animals were then euthanized by exsanguination, blood was collected from the inferior cava vein and placed into heparinized tubes. After separating a blood sample for white blood cell counting, the remaining blood was centrifuged and the plasma stored at -80°C for cytokine analysis. Tumors were dissected and weighed for prediction of cachexia index. Epididymal and retroperitoneal fat were identified, extracted and weighed. The soleus skeletal muscle was dissected, weighed, and half sectioned. One portion prepared for histological analysis (fiber cross sectional area assessment) while the other portion was frozen at -80°C for further analysis of oxidative stress (total, reduced and oxidized glutathione) and oxidative damage parameters (advanced oxidation protein products, malondialdehyde, and total lipid hydroperoxides) as well as for determination of mRNA expression of FBXO32 and mTOR genes.
Cachexia index

The cachexia index was determined considering the body weight gain of control rats, the body weight loss and tumor mass in tumor-bearing rats, according to the following equation:

\[
\text{Cachexia index} = \left( \frac{\text{Initial body weight} - \text{final body mass} + \text{mass of the tumor} + \text{control weight gain}}{\text{initial body mass} + \text{mass gain control group}} \right) \times 100 \] (Guarnier et al. 2010).

Cachexia index is presented in % of body weight changes of tumor-bearing rats related to control rats’ body weight changes.

Plasma cytokines assay and blood white cell counting

Plasma tumor necrosis factor-alpha (TNF-α) and interleukins 6 (IL-6) and 10 (IL-10) were measured using commercial ELISA kits from (Affymetrix eBioscience®, San Diego, USA). White blood cell counting and differentiation were performed using an automated counter from BD® Accuri C6.

Oxidative stress and oxidative damage analysis

Frozen soleus muscle samples were homogenized in ice-cold 50 mM sodium phosphate pH 7.0 and further centrifuged at 10,000 x g during 10 min at 4°C. Reduced (GSH) and oxidized glutathione (GSSG) were determined by the method of Rahman et al. (2006) using a 5% phosphoric acid muscle homogenate. For the assessment of oxidative damage, the supernatants were utilized for the determination malondialdehyde (MDA) and total lipid hydroperoxides, as parameters of lipid peroxidation, by the method of (Spirlandeli et al. 2014) and (Costa 2006), respectively. Advanced oxidation protein products (AOPP) were determined by the method described by (Witko-Sarsat et al. 1996).

Muscle histological analysis
For optical microscopy analysis, one portion of soleus muscle was fixed in 4% formaldehyde for 24 hours, dehydrated with graded ethanol and embedded in paraffin blocks, following routine procedures. Semi-thin sections (5µm) were cut in a microtome, applied on silane-coated slides and deparaffinized. Pieces were stained in hematoxylin and eosin, images captured on an optical microscope at a magnification of 200x, and the cross-sectional area of muscle fibers quantified (~1980 fibers per group) using Image-Pro Plus® 23 4.5 program (Media Cybernetics, Rockville, USA).

**Determination of mRNA expression**

RNA was isolated from 50 mg of frozen soleus muscle using a RiboPure Kit (Ambion, part number AM 1924, USA) according to the manufacturer’s instructions. Total RNA was quantified by spectrophotometry at OD 260/280 (NanoDrop2000c, Thermo Scientific, USA). An additional DNase I treatment (DNA-free Kit, Ambion, part number AM1906, USA) was performed to remove contaminating genomic DNA from the isolated RNA. cDNA was synthesized from 1000 ng of total RNA using a High-capacity cDNA Reverse Transcription Kit (Applied Biosystems, part number 4374966, USA). Quantitative PCR was performed using the ViiA7 Real-Time PCR System (Applied Biosystems, USA). The following Taqman® Gene Expression assay (Applied Biosystems, USA) were used in this study: FBXO32 (F-box protein 32) Rn00591730_m1; mTOR (mechanistic target of rapamycin (serine/threonine kinase) Rn00571541_m1. All amplification reactions were performed in triplicate and beta actin was used as reference gene to normalize the reactions. Beta-actin was used as endogenous reference gene. The relative expression was determined by the $2^{-\Delta\Delta CT}$ method.

**Statistical analysis**
All data showed normal distribution, as confirmed by the Shapiro-Wilk test, and reported as mean ± standard deviation. Student t test was used to test differences in cachexia index and tumor mass between tumor-bearing and tumor-bearing exercised. ANOVA for repeated measures, followed by Tukey’s post-hoc test, was used to analyze differences within- and between-groups regarding maximal strength test. Multifactorial ANOVA followed by Tukey’s post-hoc test was used to analyze differences between-groups for remaining variables. The level of significance was set at alpha<0.05.

Results

Table 1 shows the weight gain, cachexia index, final tumor weight, retroperitoneal plus epididymal fat tissue weight, and soleus muscle weight for all studied groups. Twelve days after Walker-256 tumor implantation, the rats presented a significant reduction of total body weight compared to the controls. On the other hand, RET significantly prevented body weight loss and attenuated cachexia index, as observed in the tumor-bearing exercised group when compared to the tumor-bearing group. Animals from tumor-bearing group showed a tendency for lower soleus weight, although differences among groups do not have statistical significance. The content of fat tissue in retroperitoneal and epididymal regions was significantly reduced in tumor-bearing group when compared to controls. Twelve-days of tumor inoculation were not enough to promote decreased food intake in tumor-bearing and exercised rats compared to controls.

*Insert Table 1 here*

Figure 2 presents the evolution of muscle maximum strength over 52 days of experimental protocol for all groups. Maximum strength increased significantly from day 0 to day 28 (before tumor implantation) and followed increased until day 52 (after
tumor implantation) in RET groups (exercised and tumor-bearing exercised). Significant reduction of maximum strength was observed after implantation of Walker-256 tumor in tumor-bearing animals comparatively to control. In contrast, as suggested by the results of tumor-bearing exercised, RET was able to prevent loss of muscle maximum strength induced by walker-256 tumor (Figure 2).

*Insert Figure 2 here*

The analysis of soleus muscle fibers cross sectional areas revealed a muscular atrophy in tumor-bearing animals when compared to control. RET however, was able to mitigate this tumor-induced muscle loss, as observed in tumor-bearing exercised animals (Figure 3).

*Insert Figure 3 here*

Data from muscle oxidative stress and oxidative damage markers are presented in table 2. Twelve-days after tumor implantation, an enhancement of muscular oxidative damage was evident in tumor-bearing animals, as demonstrated by the increase of malondialdehyde (39% comparatively to control group) and lipid hydroperoxides (76% comparatively to control group). The levels of advanced oxidation protein products did not show significant differences among groups. However, in tumor-bearing exercised animals, the muscular levels of malondialdehyde and lipid hydroperoxides were significantly reduced comparing to tumor-bearing animals. Regarding the muscular markers of oxidative stress, it was observed a significant decrease in reduced glutathione levels and reduced glutathione/oxidized ratio in tumor-bearing animals when compared to control.

*Insert Table 2 here*
Total and differential counting of leukocyte number and plasma interleukins levels are described in table 3. As observed, the total number of leukocytes increased ~1.5-fold in tumor-bearing comparatively to control. This was due in a large degree to the great enhancement of neutrophils in the blood stream. However, the increased leukocyte number induced by tumor cell inoculation was prevented by RET. Tumor implantation also promoted increased (P<0.05) systemic TNF-α, IL-6 levels and decreased (P<0.05) IL-10/TNF-α ratio, which was also attenuated by RET. The significant increase of the anti-inflammatory mediator IL-10 and IL-10/TNF-α ratio plasma levels in exercised groups (exercised and tumor-bearing exercised) comparatively to both sedentary ones (control and tumor) must be noted.

*Insert Table 3 here*

Analysis of quantitative Real-Time PCR data (Figure 4) showed no difference in the FBXO32 mRNA expression among the four experimental groups. However, a significant decrease of mTOR mRNA expression was observed in tumor-bearing when compared to C. RET was not able to prevent the decreased mTOR gene expression induced by tumor implantation.

*Insert Figure 4 here*

Discussion

The obtained results clearly show that RET performed prior and during tumor growth attenuates cachexia development and prevents the occurrence of muscle wasting with loss of maximal muscular strength in Walker-256 tumor-bearing rats. Moreover, the protocol also attenuates the tumor-induced systemic pro-inflammatory condition with muscle OS and muscle damage.
Our data supported that Walker-256 tumor growth was able to induce cancer cachexia development. We demonstrated that Walker-256 tumor cells inoculation favors catabolic state verified by loss of total body weight, muscle wasting and expressive reduction in peripheral fat tissue. Although no gross modification was detected on soleus muscle weight, our data demonstrated 49% and 45% of decrease in tumor-bearing soleus muscle cross-sectional area and also adipose tissue content, respectively, when compared to control group. In addition, skeletal muscle wasting was clearly associated to loss of muscle strength. This catabolic state appears to be inherent to systemic organic response against tumor growth, as demonstrated by elevated circulating TNF-α, IL-6, IL-10/TNF-α ratio and leukocytes counting. The skeletal muscle catabolic scenario is also intrinsic to local oxidative damage and oxidative stress observed in this tissue. All these together demonstrate that 12-days of Walker-256 tumor cells inoculation reproduce many of cachectic syndrome characteristics. It is known that cancer-induced metabolic disruption increases energy expenditure, promotes systemic inflammation and enhances OS in several organs and tissues, favoring the pathways of protein degradation, particularly in skeletal muscle (Guarnier et al. 2010). Skeletal muscle wasting might be mediated by excessive interleukins production, either by host or tumor cells, such as IL-6, IL-1, and TNF-α that are considered to be key-drivers of the systemic inflammation cascade (Balkwill and Mantovani 2012), triggering catabolism and impairment of anabolic pathways, which promotes muscle wasting (Argilès et al. 2014). However, this inflammatory signaling doesn’t seem to produce inflammatory infiltrate in amount enough to produce oxidative modifications on muscle proteins, since AOPP quantification was not significant among groups. In addition, and according to this scenario, our data demonstrated that 12-days implanted Walker-256
tumor decreased mTOR signaling, unlikely, tumor-bearing sedentary rats did not presented increased FBXO32 level when compared to control group.

In the last few years, studies have addressed the potential effects of exercise on cancer cachexia (Bacurau et al. 2007; Lira et al. 2008; Padrão et al. 2015; Khamoui et al. 2016). Most of them have focused on endurance exercise training, demonstrated as a safe strategy to prevent and attenuate cancer-induced perturbations in different organic systems and body compartments. RET is known to promote physiological and structural changes in skeletal muscle, demonstrated in our study by increased cross-sectional area and muscle strength in exercised when compared to control group. These results proved the efficacy of our climbing protocol on generate positive muscle adaptation as that demonstrated in RET performed by humans. Remarkably, RET performed 6-weeks prior and 12-days after Walker-256 tumor cells inoculation was able to mitigate body weight loss and reduce cachexia index, preventing characteristic muscle wasting and muscular strength loss. The infiltration of inflammatory cells inherent to the interaction area between tumor and host cells releases mediators capable of eliciting a local inflammatory response with systemic and local skeletal muscle repercussions (Grivennikov et al. 2010). In contrast, RET protective effects appears to be inherent to the pro-inflammatory and muscle oxidative stress-attenuating effects induced by tumor development. Donatto et al. (2013) demonstrated that RET is able to down-regulate chronic inflammation induced by Walker-256 tumor cells inoculation, in a similar way to that previously demonstrated with endurance training (Bacurau et al. 2007; Lira et al. 2008). Studies also have demonstrated that IL-10 have pivotal role on anti-inflammatory response, acting as immunosuppressant of IL-1β and TNF-α interleukins (Lira et al. 2009; Schottelius et al. 1999; Pretolani 1999; Frajacomo et al. 2015). Lira et al. (2009) demonstrated the IL-10/TNF-alpha ratio has been used to reflect an anti-
inflammatory state on exercise protocols. Additionally, in an attempt to maintain the muscular homeostasis, RET could induce adaptation of antioxidant mechanisms to reduce damage, leading the system to coordinately work to resist the redox imbalance in cells and reduce oxidative damage (Gomez-Cabrera et al. 2005; Kosmidou et al. 2002) demonstrated that improvement of antioxidant system adaptation generated by RET corroborates to diminished systemic inflammation (Kosmidou et al. 2002). In our study, we demonstrated that RET inhibits oxidative damage induced by tumor development and increased anti-inflammatory state demonstrated by elevated IL-10 and IL-10/TNF-α ratio in exercised and tumor-bearing exercised groups, compared to tumor-bearing and control groups. Moreover, the generation and release of anti-inflammatory cytokines stimulated by muscular contraction exerts inhibitory effect on pro-inflammatory interleukins production (in this case, TNF-alpha and IL-1) (Schindler et al. 1990). Frajacomo et al. (2015) demonstrated that aerobic exercise promotes antipre-neoplastic effects in the colon cells by enhancing IL-10 activity. In this sense, RET-induced increased IL-10 secretion and enhanced antioxidant muscular activity. This response may improve local and systemic anti-inflammatory condition, leading to prevention of catabolic state, which would act as mechanisms underlying proteolysis and lipolysis conditions. Tidball and Welc (2015) demonstrated the involvement of anti-inflammatory cytokines associated with concomitant activation of the IGF-1 muscle pathway as a potent enhancer of tissue regeneration. Important to note, however, that RET protocol proposed in our study was unable to up-regulate mTOR mRNA level or down regulate FFOX32 signaling. It seems contradictory, since these are considered important genes for anabolism and catabolism pathways signaling, respectively (Argilés et al. 2014). Atrogin-1 was already demonstrated to have this peak of expression, in this tumor model of cachexia, 5 days after tumor inoculation, being down regulated after 10
days (Guarnier et al. 2010). In this sense, the lack of difference on FBOX32 expression demonstrated here was already expected. However, these interpretation of the results is not so simple, since exercise in cancer-induced cachexia can differ between exercise type, skeletal muscle group and cachexia-inducing model studied. In addition, we consider important to say that the anabolic/catabolic signaling cascade is complex and involves several genes and protein signaling.

Finally, it should be reinforced here that, even with the lack of changes in muscle soleus weight, tumor inoculation and RET promoted decreased and increased soleus fiber muscle cross-sectional area, respectively. It is important to note that the wet weight of the muscle cannot be used alone as a fine muscle loss or atrophy parameter. In some cases, the weight is not a parameter sensitive enough to determine presence of edema, here probably present in tumor-bearing rats muscle, and widely described as pro-inflammatory condition (Kumar et al. 2016). In addition, our data demonstrated that RET was able to preserve muscle strength in tumor-bearing exercised animals compared to tumor-bearing animals. Once implanted, tumor cell may target skeletal muscle, since it provides immediate energy through muscle glycogen (Zois and Harris 2016). In this scenario, skeletal muscle wasting may reduce the ability to generate power, which may decrease muscle function and strength (Ardies 2002). At the present, it has been demonstrated that the dynapenia in humans is a hallmark as important as muscle wasting alone for cancer prognosis (Fearon et al. 2012). In this perspective, the preservation of muscle mass is essential for maintaining muscle function that may preserve quality of life for cancer patients (Argilés et al. 2013). Thus, our study demonstrated that RET is a promising strategy that can contribute to the prevention of muscle wasting-mediated strength loss in Walker-256 tumor-bearing rats, a strategy that is poorly tested in humans with cancer.
In conclusion, our data demonstrated that RET preformed prior and after tumor implantation prevents cachexia development, mostly characterized by impaired body weight gain and muscle wasting. In addition, loss of maximal muscular strength induced by tumor was also attenuated by means of inflammatory state, muscle oxidative stress and muscle damage prevention. These data are new and demonstrated a potential therapeutic effect of RET on preventing cancer cachexia.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Reference


whole-body protein synthesis and growth rate in the cancer-bearing rat. Cancer Res. 46(11), 5649-5654. PMID: 3756911.


Table 1. General characteristics of control (C), tumor-bearing (T), exercised (E) and tumor-bearing exercised (TE) rats.

<table>
<thead>
<tr>
<th></th>
<th>C(n = 9)</th>
<th>T(n = 9)</th>
<th>E(n = 9)</th>
<th>TE(n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain after inoculation (g/12 days)</td>
<td>13.0±1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-12.2±2.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.7±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2±2.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Final tumor weight (g)</td>
<td>-</td>
<td>8.2±1.2</td>
<td>-</td>
<td>8.4±1.1</td>
</tr>
<tr>
<td>Cachexia index (%)</td>
<td>-</td>
<td>9.5±1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>5.0±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soleus muscle weight (g)</td>
<td>0.35±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>∑ fat weight (g)</td>
<td>6.9±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.4±0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.7±1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
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</tbody>
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Data are presented as mean and standard deviation. a, b Different letters in the same row differ (P<0.05; ANOVA with post-hoc test of Tukey). ∑ fat weight: retroperitoneal plus epididymal fat
Table 2. Muscle oxidative stress and oxidative damage markers in control (C), tumor-bearing (T), exercised (E) and tumor-bearing exercised (TE) rats.

<table>
<thead>
<tr>
<th></th>
<th>C (n = 9)</th>
<th>T (n = 9)</th>
<th>E (n = 9)</th>
<th>TE (n = 10)</th>
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<tbody>
<tr>
<td>MDA (µmol/g protein)</td>
<td>109.1±5.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>119.36±9.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>75.25±9.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Lipidhydroperoxides (µmol/g protein)</td>
<td>367.5±39.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>646.1±29.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>469.3±65.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>305.7±68.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>AOPP (µmol chloramine-Tequivalents/ g protein)</td>
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<td>64.2±8.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>75.1±9.2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>GSH (µmol/g protein)</td>
<td>592.2±54.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>532.5±37.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>149.3±17.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>130.8±9.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>3.8±0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as mean and standard deviation. a,b Different letters in the same row differ (<i>P</i>&lt;0.05; ANOVA with post-hoc test of Tukey). MDA: malondialdehyde, AOPP: advanced oxidation protein products, GSH: reduced glutathione, GSSG: oxidized glutathione.
Table 3. Leukocyte number and differential counting of plasma inflammatory mediators and plasma cytokines levels for control (C), tumor-bearing (T), exercised (E) and tumor-bearing exercised (TE) rats.

<table>
<thead>
<tr>
<th>Blood white cell counting</th>
<th>C (n = 9)</th>
<th>T (n = 9)</th>
<th>E (n = 9)</th>
<th>TE (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of leukocytes (cu mm x 10^3)</td>
<td>8.2±3.5^a</td>
<td>20.8±3.4^b</td>
<td>8.5±1.8^a</td>
<td>12.8±4.7^a</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>74.3±6.5^a</td>
<td>49.1±9.3^b</td>
<td>70.0±9.5^a</td>
<td>41.1±13.9^b</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>16.3±7.4^a</td>
<td>43.5±9.0^b</td>
<td>20.2±10.5^a</td>
<td>50.5±24.0^b</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>8.8±3.4</td>
<td>7.4±4.6</td>
<td>9.8±2.4</td>
<td>8.3±4.1</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.0±1.7</td>
<td>0.5±0.2</td>
<td>0.5±0.2</td>
<td>0.5±0.3</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.0±0.6^a</td>
<td>8.1±2.2^b</td>
<td>0.8±0.6^a</td>
<td>4.4±1.8^c</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.6±0.2^a</td>
<td>20.0±6.8^b</td>
<td>3.2±1.1^a</td>
<td>4.1±1.1^a</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>277.2±90.8^a</td>
<td>167.8±26.2^a</td>
<td>547.7±171.7^b</td>
<td>498.9±205.5^b</td>
</tr>
<tr>
<td>IL-10/TNF-α ratio</td>
<td>272.78±110.5^a</td>
<td>21.77±7.3^b</td>
<td>601.87±244.3^c</td>
<td>139.5±66.5^ab</td>
</tr>
</tbody>
</table>

Data are presented as mean and standard deviation. a,b Different letters in the same row differ (P<0.05; ANOVA with post-hoc test of Tukey).
Figure captions

Figure 1. Schematic view of study design.

Figure 2. Evolution of the maximum load achieved in 3 maximal strength tests over 52-days of experiment of control (C), tumor-bearing (T), exercised (E) and tumor-bearing and exercised (TE) groups. Data are presented as mean and standard deviation. a,b Mean values followed by different letters were significantly different between groups at the same time; *indicates a significant difference of each group in relation to previous session (P<0.05 by ANOVA for repeated measures for within- and between-group).

Figure 3. H&E-stained muscle soleus sections (A), cross sectional area distribution (B) and median and quartile range (25-75) of myofibers (C) determined for control (C), tumor-bearing (T), exercised (E) and tumor-bearing exercised (TE) groups. a,b different letters differ (P<0.05; ANOVA with post-hoc test of Tukey).

Figure 4. mTOR and FBXO32 genes mRNA expression for control (C), tumor-bearing (T), exercised (E) and tumor-bearing exercised (TE) groups. Data are represented as mean and standard deviation. a,b different letters differ (P<0.05; ANOVA with post-hoc test of Tukey).
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