Original Article

Effect of physical activity in an enriched environment on the skeletal muscle of mice with cachexia associated with cutaneous melanoma

Efeito da atividade física em ambiente enriquecido sobre o músculo esquelético de camundongos com caquexia associado ao melanoma cutâneo

Karen Layane dos Santos[1]
Tayrine Resende de Oliveira[2]
João Vitor Nunes Lopes[3]
Alex Sander Freitas[4]
Magda Mendes Vieira[5]
Berenilde Valéria de Oliveira Sousa[6]
Mariana Rocha Alves[7]
Vinicius Dias Rodrigues[8]

[1]Corresponding author. Universidade Estadual de Montes Claros (Montes Claros). Minas Gerais, Brazil. kklayane@yahoo.com.br
[2]Ambulatório de Neurociências do Exercício, Hospital Aroldo Tourinho (Montes Claros). Minas Gerais, Brazil. tayrineoliveirauni@gmail.com
[3]Faculdade Integradas do Norte de Minas (Montes Claros). Minas Gerais, Brazil. joaollopes@outlook.com.br
[4-6]Universidade Estadual de Montes Claros (Montes Claros). Minas Gerais, Brazil. alexsanderfreitas3@gmail.com, magdamendesvieira@hotmail.com, berenilde.valeria7@gmail.com
[7]Faculdade Verde Norte (Mato Verde). Minas Gerais, Brazil. marianarochaalves13@gmail.com
[8]Universidade Estadual de Montes Claros (Montes Claros). Minas Gerais, Brazil. viniciuslabex@hotmail.com

ABSTRACT | OBJECTIVE: The objective of this study was to verify the effects of physical activity in an enriched environment on histomorphometry of the quadriceps femoris muscle of C57BL / 6 mice with cachexia associated with the cutaneous melanoma tumor model. **METHODS:** Female mice of the C57BL / 6 strain were used and were randomly assigned to two groups: CRC control (n = 11), which did not perform any type of intervention, the second group was the experimental one (CRC-ATF) (n = 15), who performed organized physical activity in an enriched environment 60 cm long, 30 cm wide and 45 cm high. Tumor induction of B16-F10 cells of the cutaneous melanoma lineage occurred in all animals in this study. After ten days of tumor induction, all animals already presented with cachexia, so the experimental group started physical activity in an enriched environment lasting 30 minutes with intervals of 48 hours. 26 days after the beginning of the intervention, the surviving animals were euthanized and the quadriceps femoris muscle was collected for histomorphometric analysis. **RESULTS:** Analyzing the intervention performed, we noticed that the CRC-ATF group had a larger muscle fiber area than the CRC group, but this result showed no significant difference ($p \leq 0.05$) between the groups. **CONCLUSION:** The results show that it is possible to preserve the muscular structure, as the best results were found with animals that participated in physical activity in an enriched environment.

KEYWORDS: Physical fitness. Skeletal muscle tissue. Rodents. Cancer. Neoplastic.

RESUMO | OBJETIVO: O objetivo deste estudo foi verificar os efeitos da atividade física em ambiente enriquecido sobre histomorfometria do músculo quadríceps femoral de camundongos C57BL/6 com caquexia associada ao modelo tumoral singênico de melanoma cutâneo. **MÉTODOS:** Foram utilizados camundongos fêmeas da linhagem C57BL/6 que foram distribuídos aleatoriamente em dois grupos: controle CRC (n=11), que não realizou nenhum tipo de intervenção, o segundo grupo foi o experimental (CRC-ATF) (n=15), que realizou atividade física organizada em ambiente enriquecido de 60 cm de comprimento, 30 cm de largura e 45 cm de altura. Ocorreu indução tumoral de células B16-F10 da linhagem de melanoma cutâneo em todos os animais desse estudo. Após dez dias da indução tumoral, todos os animais já apresentavam quadro de caquexia, assim o grupo experimental iniciou a atividade física em ambiente enriquecido com duração de 30 minutos com intervalos de 48 horas. Após 26 dias do início da intervenção, os animais sobreviventes foram eutanasiados e foi realizada a coleta do músculo quadríceps femoral para análise histomorfométrica. **RESULTADOS:** Analisando a intervenção realizada, percebemos que o grupo CRC-ATF apresentou a área da fibra muscular maior que o grupo CRC, mas esse resultado não mostrou diferença significativa ($p \leq 0,05$) entre os grupos. **CONCLUSÃO:** Os resultados mostram que é possível preservar a estrutura muscular, pois os melhores resultados foram encontrados com os animais que participaram da atividade física em ambiente enriquecido.

PALAVRAS-CHAVE: Aptidão física. Tecido muscular esquelético. Roedores. Câncer. Neoplasia.

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Introduction

Melanoma is a malignant neoplasm that originates from melanocytes, cells that produce melanin[1]. Melanoma represents about 4% of all skin tumors, and despite this low representativeness it is considered to be the most important medical skin cancer, due to the incidence and mortality rate, and it can be very aggressive, as it has a high capacity to perform lymphatic and hematogenous metastases[2]. A negative prognosis of cancer is the manifestation of cachexia, a multifactorial syndrome that is characterized by the loss of skeletal muscle and adipose tissue[3].

Cancer-associated cachexia is a complex syndrome, characterized by a debilitating state, where involuntary loss of body weight, muscle loss and metabolic changes occur in 50-80% of advanced malignant tumors, representing about 20% of deaths from cancer patients[4]. These factors benefit the tumor tissue in the dispute over energy sources with other cells in the body[5,6]. In this same aspect, the level of body fat undergoes considerable reduction due to the increase in lipolysis and oxidation of lipids with a decrease in the amount of circulating lipids, supposedly due to the increased use of fatty acids for the supply of energy and tumor growth[7].

As an alternative treatment in the preservation of these functions, several studies report physical activity in order to assist in maintaining weight and neuromuscular functions and in combating states of fatigue and cachexia[8]. Because, according to Al-Majid and McCarthy[9], physical activity has the ability to induce changes both in the synthesis pathway and in that of protein degradation, both pathways are related to the loss of muscle tissue in cancer patients.

Physical activity is able to alter the immune functionality of healthy individuals, possibly due to changes in the concentrations of some cytokines. As cachexia is predominantly caused by cytokines produced by the host's immune system or by the tumor, physical activity can be a type of intervention to be used in the treatment of cancer[10].

Thinking of better experimental exercise or physical activity strategies with mice for the intervention of cachectic conditions, Aguiar Jr and Pinho[11], report that studies with models of physical activity in an enriched environment have important results in the general health of rodents, but still, they do not have models experimental studies with cachexia associated with cancer that show such benefits, mainly due to the skeletal muscle structure. Thus, the present study aimed to verify the effects of physical activity in an enriched environment on histomorphometry of the quadriceps femoris muscle of C57BL/6 mice with cachexia associated with the syngeneic tumor model of cutaneous melanoma.

Methods and materials

Study characterization and ethical care

The present study was analytical, prospective and with a quantitative approach. The work was submitted to analysis by an ethics committee on animal experimentation and welfare (CEEBEA / Unimontes) and had a favorable ruling for execution (131/2017). 38 female C57BL / 6 mice were used and were randomly assigned to two groups. The first group was the control (CRC) (n = 11), did not perform any type of intervention, the second group was the experimental (CRC-ATF) (n = 15), performed physical activity in an enriched environment. Tumor induction of B16-F10 cells of the cutaneous melanoma lineage occurred in all animals in this study.

Before, an experiment was carried out to define the characterization of cachexia in the syngeneic model of cutaneous melanoma using a murine melanoma lineage B16-F10, for this assay 12 animals were used[12].

Tumor induction

The C57Bl/6 animals are indicated in experiments for tumor implantation, with emphasis on the singenic model of cutaneous melanoma using the murine melanoma lineage B16-F10. First, the C57Bl/6 mouse was immobilized and subjected to trichotomy of the dorsal region, close to the neck. Then, the absence of painful reflex sensitivity was verified. After asepsis with iodized alcohol, the animals were inoculated with 5x105 cells of murine melanoma B16-F10, resuspended in 50µL, in the dorsal subcutaneous region, close to the base of the neck (flank). The inoculation of this quantity of viable cells in the subcutaneous region has the capacity to complete a mitotic cycle in 24 hours and to develop the tumor within 3-4 days[12].

Experimental physical activity format

To avoid compromising the experimental protocols, there were two 30-minute sessions of physical activity (PA) in an enriched environment before tumor induction with the aim of familiarizing the mice in the experimental group.

The PA was organized in an enriched environment 60 cm long, 30 cm wide and 45 cm high. This environment was combined with seesaw, wheels, balls and tunnels, all animals were together for 30 minutes per session. There were seven sessions at 48 hour intervals. During the sessions, the animal's movement, speed and displacement were not controlled[13]. The animals had free access to filtered water and balanced feed (Purina-Labina®) at the place of accommodation in the bioterium.

Diagnosis of cachexia

Cachexia related to cutaneous melanoma (CM) was established as soon as mice with the syngeneic MC model showed a loss of at least 5-10% of body weight (disregarding the weight of the tumor) during tumor progression[14]. A linear regression equation was used to define the relationship between tumor volume and tumor weight. The measurements were performed 12 days after the inoculation of tumor cells in a sample of 12 animals, in order to calculate the tumor weight throughout the experiment. Each day, a mouse was euthanized and the tumor volume (mm3) and mass (g) were measured to obtain tumors with different volume[15]. At the end of the experiment, data were obtained from different tumor stages and the relationship between mass and volume was defined in the linear equation (R2 = 0.9892). Once the tumor weight measurement was defined, these values were subtracted from the body weight measurement of each animal per day. Thus, after performing the cachexia diagnostic experiment, the tenth day was established for the diagnosis of cachexia after tumor induction for all animals, thus, the experimental group started the PA intervention, at each session they had 48 hours of break for the next session. 48 hours after the seventh session, the surviving animals were euthanized.

Instruments and procedures for assessing body composition

26 days after the beginning of the experiment, all 26 animals of the control and experimental group were anesthetized with ketamine/xylazine (75 mg/kg and 5 mg/kg weight, respectively) and euthanized by cervical dislocation[16], to perform the collection of muscle tissues skeletal structures of the quadriceps femoris. The collected tissues were weighed using an analytical precision digital scale (A. Cientifica EEQ9003E)[17].

Instrument and procedures for histological analysis of the quadriceps femoris muscle

Biological samples collected (quadriceps femoris muscle) were weighed individually, sectioned and placed part in 4% paraformaldehyde fixing solution (p: v), 4h fixation time, at room temperature and part in RNA holder and tissue tek for freezing a - 80 ºC. The samples fixed in 4% buffered formalin solution were embedded in paraffin and were subjected to microscopic cuts to perform morphometric studies. Skeletal muscle tissues fixed in formalin and implanted in paraffin from the right hind limbs (quadriceps femoris) were submitted to 7 µm cuts and marked in Hematoxylin-Eosin (HE). Sections of the skeletal muscles were made at 90o and 180o for the longitudinal axis of the muscle fibers. At least three distinct randomly indicated microscopic fields of skeletal muscle tissues per animal were photographed. Muscle sections were photographed using an Olympus BX50 microscope (Olympus Corp., JAP). The area of the transversal muscle fiber was measured using the Image J software (imagej.nih.gov). All data are expressed as the mean ± SEM. Three slides of the muscle tissue of each animal were analyzed and photographed, on the same slide, 5 different locations were photographed.

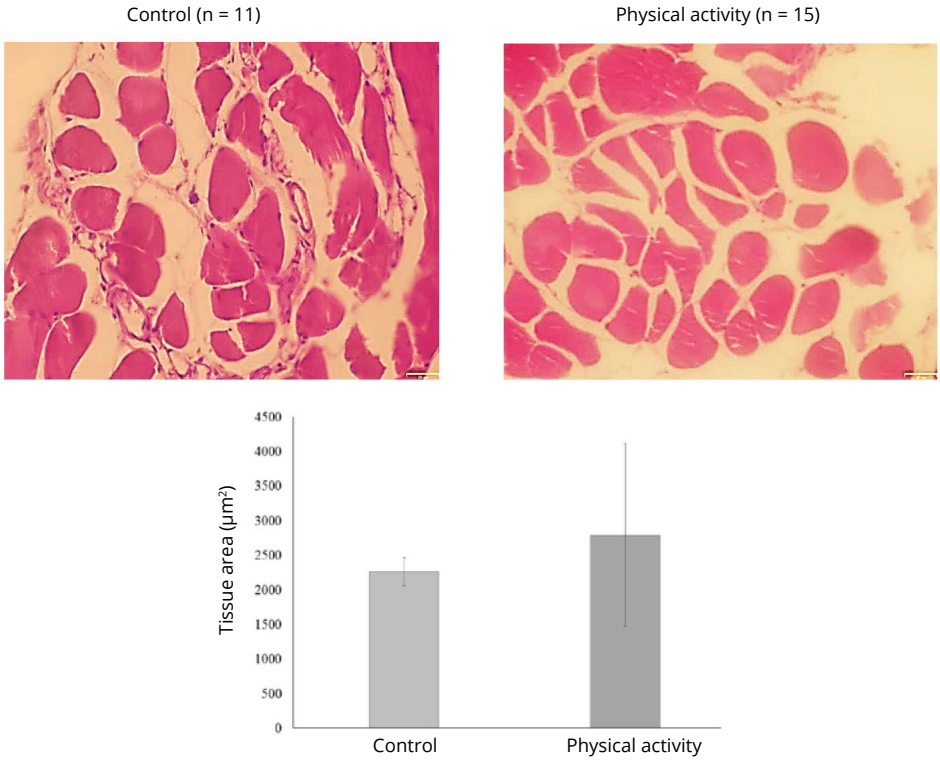
Statistical analysis

All data collected were statistically analyzed using SPSS (Statistical Package for the Social Sciences) version 20.0. The confidence level adopted in all analyzes was set at 95% ($p < 0.05$). The Shapiro-Wilk test was performed to verify normality, then the independent Student T test was selected for inferential analysis of the dependent variables.

Results

The results in Fig. 1 show the histomorphometric analysis of the fiber area of the quadriceps femoris muscle of the right rear limb of C57BL/6 mice from the murine syngeneous tumor model of cutaneous melanoma, the inferential analysis using the independent Student T test does not show any significant difference. ($p < 0.05$) between the groups, but the average muscle fiber area of the CRC-ATF was greater than that of the control group.

Figure 1. Analysis of the fiber area of the quadriceps femoris muscle of the right rear limb of C57BL / 6 mice from the murine syngeneous tumor model of cutaneous melanoma and with the occurrence of cachexia submitted to physical activity in an enriched environment and controls.



Discussion

In a study by Rodrigues et al.[13] using several models, among them the physical activity model in an environment enriched with healthy female C57BL/6 mice, the results showed that there were contributions in muscle strength, body composition, and histomorphometry. However, this difference was not significant compared to the control group.

The state of muscle atrophy in cachexia associated with cancer occurs due to the intense catabolism activity in the body of the tumor "host" causing an inflammatory response caused by the increase in cytokines (TNF-α and IL-6). This inflammation establishes a new prioritization in the metabolism of macronutrients, promoting the increase of oxidative stress and the ubiquitin-proteasome proteolytic system, both responsible for the degradation of proteins, thus causing a decrease in protein synthesis, used as a source to meet the increased metabolic demands in the host caused by the presence of the tumor[5,18,6,3].

The average muscle fiber size of the animals in the innervation group was greater than in the group without intervention, despite the not significant difference, the results are expressive, the results show that it is probably possible to preserve the muscle structure, as the best results were found with the animals that participated in physical activity in an enriched environment, probably due to reducing the action of TNFα and IL-6 and increasing the activity of the enzymes mustase superoxide (SOD), glutathione perioxidase (GPx) and mitochondrial Mn-SOD, causing an action protective against cell damage of reactive oxygen species (ROS) and increasing the rate of protein synthesis[19]. Corroborating with Ballarò et al.[20], moderate exercise is able to mitigate the decrease in muscle mass and prevent the loss of muscle strength, as this situation was associated with reduced levels of reactive oxygen species, in this work, oxidative stress was investigated in the presence and absence of moderate aerobic training in albino cachectic mice Balb/c with colorectal carcinoma.

Necropsy findings from human beings, in various muscles, revealed that after cases of cachexia, the fibers presented atrophy predominantly II with occasional angular fibers; presenting in large groups, greater in lower limbs[21].

Although the findings are interesting, the absence of a control group and the small sample size are limiting to other questions, however, the results presented here are of great scientific importance. It becomes necessary to propose new research to elucidate the gaps left here.

Conclusions

The results of this study show that there were no significant changes in the fiber area of the quadriceps femoris muscle in cachectic mice that performed physical activity in an enriched environment when compared to the control group.

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Author contributions

Santos KL, Lopes JVN and Sousa BVO carried out the experiments in the vivarium. Oliveira TR outlined the statistical method necessary for the study. Freitas AS and Vieira MM performed the histological analyses of the muscle tissue samples. Alves MR and Rodrigues VD supervised and coordinated the research.

Competing interests

No financial, legal or political competing interests with third parties (government, commercial, private foundation, etc.) were disclosed for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.).

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