UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Vinicius Dias Rodrigues

Efeitos do treinamento resistido e da atividade física em camundongos C57BL/6 com caquexia associada ao modelo tumoral singênico de melanoma cutâneo.

Montes Claros - MG
2018

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Tese apresentada ao Programa de Pós-graduação em Ciências em Saúde da Universidade Estadual de Montes Claros, como parte das exigências para a obtenção do título de Doutor em Ciências da Saúde.

Área de Concentração: Mecanismos e Aspectos Clínicos das Doenças.

Orientador: Prof. Dr. Alfredo Maurício Batista de Paula

Coorientadora: Prof. Dra. Ludmilla Regina de Souza

Coorientador: Prof. Dr. Renato Sobral M. Júnior

Montes Claros – MG
2018

Rodrigues, Vinicius Dias.

R696e Efeitos do treinamento resistido e da atividade física em camundongos C57BL/6 com caquexia associada ao modelo tumoral singênico de melanoma cutâneo / Vinicius Dias Rodrigues. – 2018.

132f. : il.

Inclui Bibliografia.

Tese (doutorado) - Universidade Estadual de Montes Claros - Unimontes, Programa de Pós-Graduação em Ciências da Saúde/PPGCS, 2018.

Orientador: Prof. Dr. Alfredo Mauricio Batista de Paula.

Coorientadora: Profa. Dra. Ludmilla Regina Souza.

Coorientador: Prof. Dr. Renato Sobral Monteiro Júnior.

1. Treinamento resistido. 2. Atividade física. 3. Atividade física espontânea. 4. Validação. 5. Câncer. 6. Caquexia relacionada ao câncer. 7. Modelo melanoma cutâneo singênico. 8. Sobrevida. I. Paula, Alfredo Mauricio Batista de. II. Souza, Ludmilla Regina. III. Monteiro Júnior, Renato Sobral. IV. Universidade Estadual de Montes Claros. V. Título.

Catalogação: Biblioteca Central Professor Antônio Jorge.

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UNIVERSIDADE ESTADUAL DE MONTES CLAROS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Unimontes

CANDIDATO: VINÍCIUS DIAS RODRIGUES

TÍTULO DO TRABALHO: "Efeitos do treinamento resistido e da atividade física em camundongos C57BL/6 na Caquexia associada ao modelo tumoral singênico de Melanoma cutâneo".

ÁREA DE CONCENTRAÇÃO: Mecanismos e Aspectos Clínicos das Doenças

LINHA DE PESQUISA: Etiopatogenia e Fisiopatologia das Doenças

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[X] **APROVADO(A)** [ ] **REPROVADO(A)**

Dedico este trabalho à minha família e amigos, em especial
aos meus pais, irmão e esposa que sempre compartilharam dos meus sonhos.

AGRADECIMENTOS

Chegado o final desta relevante etapa acadêmica, não poderia deixar de expressar os meus mais sinceros e profundos sentimentos de gratidão para com todos aqueles que me apoiaram, ao longo dessa caminhada, tornando possível a realização deste sonho que ora se concretiza. Deste modo deixo aqui expresso o meu agradecimento:

Primeiramente, ***a Deus***, fonte fecunda e imortal de luz.

Aos meus ***pais e irmão,*** pelo carinho, apoio e compreensão em todos os momentos da minha vida.

À minha esposa, ***Mariana***, que durante todo esse processo esteve ao meu lado com paciência e parceria. Obrigado por fazer parte da minha vida.

Ao ***Prof. Dr. Alfredo Mauricio Batista de Paula***, pela sua orientação, disponibilidade, dedicação, pela colaboração, pelo amigo que, mais uma vez demonstrou ser através da infindável ajuda e disponibilidade para minha formação profissional acadêmica.

Ao ***Prof. Dr. Renato Sobral Monteiro Júnior***, pela amizade, parceria e incentivo na minha vida acadêmica.

Aos professores (as) ***Ludmilla Regina de Souza, Sergio Henrique Sousa Santos, Wellington Danilo Soares, Ricardo Cardoso Cassilhas, Adriana Bozzi de Melo, João Marcus Oliveira Andrade, Lucyana Conceição Farias*** *e* ***Marcos Vinícius Macedo de Oliveira*** pelo imprescindível apoio e colaboração nesse trabalho.

Ao ***Programa de Pós-Graduação em Ciências da Saúde – PPGCS*** por abrir suas portas para minha capacitação acadêmico-científica.

À ***Universidade Estadual de Montes Claros – Unimontes,*** *à* ***Prefeitura Municipal de Montes Claros/MG*** e às ***Faculdades Integradas do Norte de Minas – Funorte***, pelo apoio e incentivo a minha capacitação enquanto docente destas instituições de ensino superior.

À minha amiga ***Magda Mendes Vieira*** que participou efetivamente na construção desse trabalho e dividiram comigo os momentos difíceis nessa jornada acadêmica.

Aos ***amigos*** e ***amigas*** que sempre ajudaram para construção desse sonho, o meu profundo agradecimento.

'' O importante não é vencer todos os dias, mas lutar sempre.''
(**Waldemar Valle Martins**)

RESUMO

O objetivo desse trabalho foi investigar os efeitos do treinamento resistido e da atividade física em camundongos C57BL/6 com caquexia associada ao modelo tumoral singênico de melanoma cutâneo. Para construção dessa tese foram realizados três produtos. Primeiramente foi elaborado um protótipo (modelo de utilidade) para exercício resistido com camundongos C57BL/6, em seguida foi produzido o primeiro artigo que analisou os efeitos do exercício resistido utilizando a escalada com estímulo de choque elétrico (protótipo) nas variáveis da força muscular, da composição corporal, do volume de membro, na área de fibras musculares, no número de núcleos na fibra muscular e dos níveis plasmáticos de lactato sanguíneo. Para isso, foram analisados camundongos C57BL/6 saudáveis praticantes de atividade física, exercício resistido, exercício resistido com estimulo de choque e animais sedentários. De acordo com os dados, o modelo de escada de eletroestimulação vertical apresentou efeitos agudos sobre os níveis de lactato semelhante a outros modelos experimentais de exercício resistido e atividade física espontânea. Além disso, na resposta crônica de curto prazo, a comparação entre o modelo proposto e as demais intervenções, revelou que os melhores resultados do protótipo foram obtidos no desempenho da força muscular de tração dos membros, ganho de peso dos tecidos adiposo marrom e músculo quadríceps femoral, e maior número de núcleos nas fibras musculares estriadas esqueléticas ($p < 0,05$ em todas as comparações). Posteriormente, após os achados do segundo produto, foi realizado um novo estudo (terceiro produto) com objetivo de verificar os efeitos do exercício resistido e atividade física na estrutura músculo esquelético, tecido adiposo branco e sobrevida em camundongos C57Bl/6 com caquexia cutânea relacionada ao melanoma. Intervenções do exercício resistido e da atividade física contribuíram para o aumento da força muscular, melhora na composição corporal (tecido muscular e adiposo marrom) e histomorfometria do músculo gastrocnêmico dos animais com caquexia associada ao melanoma quando as intervenções foram realizadas iniciaram antes do estabelecimento do quadro caquético. O exercício resistido realizado após o diagnóstico da caquexia não ocorreu alteração significativa na expressão genica (*MYOG, FXOB32, IGF1, TRIM63* e *PPARγ*), acreditamos que o controle de carga (intensidade e volume) deva ser modificado para melhor rendimento dessas variáveis. Em relação à sobrevida, nossos achados mostraram as intervenções propostas não proporcionaram maior sobrevida global. Assim, estudos experimentais com outros modelos metodológicos de exercício físico com camundongos portadores de melanoma cutâneo devem ser encorajados para elucidar as lacunas do corrente estudo, visto que o exercício resistido e a atividade física apresentaram benefícios diante do ganho de força muscular e aumento do músculo gastrocnêmio em animais com caquexia associada ao melanoma.

Palavras-chave: Treinamento Resistido; Atividade Física; Atividade Física Espontânea; Validação; Câncer, Caquexia Relacionada ao Câncer, Modelo Melanoma Cutâneo Sigênico, Sobrevida.

ABSTRACT

The objective of this study was to investigate the effects of resistance training and physical activity in C57BL/6 mice with cachexia associated with the syngeneic tumoral model of cutaneous melanoma. For the construction of this thesis, three products were made. First, a prototype (utility model) was developed for resistance exercise with C57BL/6 mice, and the first article that analyzed the effects of resistance exercise using electric shock stimulus (prototype) on the variables of muscle strength, body composition, limb volume, muscle fiber area, number of muscle fiber nuclei, and plasma lactate levels. For this, we analyzed healthy C57BL/6 mice practicing physical activity, resistance exercise, resistive exercise with shock stimulus and sedentary animals. According to the data, the vertical electrostimulation ladder model presented acute effects on lactate levels similar to other experimental models of resistance exercise and spontaneous physical activity. In addition, in the short-term chronic response, the comparison between the proposed model and the other interventions showed that the best results of the prototype were obtained in the performance of limb traction muscle strength, brown adipose and quadriceps muscle weight gain femoral, and greater number of nuclei in the skeletal striated muscle fibers ($p < 0.05$ in all comparisons). Later, after the findings of the second product, a new study (third product) was carried out to verify the effects of resistance exercise and physical activity on skeletal muscle structure, white adipose tissue, and survival in C57Bl/6 mice with cutaneous cachexia related to melanoma. Interventions of resistive exercise and physical activity contributed to an increase in muscle strength, improvement in body composition (muscle tissue and brown adipose tissue) and histomorphometry of the gastrocnemius muscle of animals with cachexia associated with melanoma when the interventions were performed before the establishment of the condition cachectic. The resistance exercise performed after the diagnosis of cachexia did not change significantly in the genetic expression (*MYOG, FXOB32, IGF1, TRIM63* and *PPARγ*),, we believe that the load control (intensity and volume) should be modified to better yield these variables. Regarding survival, our findings showed that the proposed interventions did not provide greater overall survival. Thus, experimental studies with other methodological models of physical exercise with mice with cutaneous melanoma should be encouraged to elucidate the shortcomings of the current study, since resistance exercise and physical activity presented benefits due to the gain of muscle strength and an increase of the gastrocnemius muscle in animals with cachexia associated with melanoma.

Key words: Resistance Training; Physical activity; Physical Activity Spontaneous; Validation; Cancer, Cancer-Related Cachexia, Cutaneous Sigmanic Melanoma, Survival.

SUMÁRIO

1 INTRODUÇÃO

1.1 Melanoma cutâneo

Nos melanócitos, células de melanina, pode ocorrer um tipo de câncer conhecido por melanoma. O processo de desenvolvimento inicia com um pequeno tumor cutâneo pigmentado sobre a pele normal, geralmente a frequência são em áreas expostas ao sol, porém metade dos casos ocorre a partir de nevos melanocitos pré-existentes (1).

Esse tipo de câncer é o responsável pela maioria das mortes por câncer de pele e diferentes dos demais acometimentos cutâneos, sua evolução ocorre de forma rápida a metástase, posteriormente dificultando a viabilidade do tratamento (2,3).

No mundo, os dados mostram preocupação, pois o melanoma maligno é o 19º câncer mais comum, com cerca de 232.000 novos casos diagnosticados por ano (4). No Brasil, teve uma incidência de 5.890 casos (2.960 casos novos em homens e 2.930 em mulheres) de melanoma cutâneo no biênio de 2014/2015 (5). Percebe-se que sua incidência tem aumentado rapidamente (4,5). Na região Sul do Brasil o crescimento de acometimentos é o maior no pais, essas variações entre os regiões parecem refletir a participação de diferentes fatores de risco (6).

O melanoma cutâneo pode ser classificado em quatro tipos distintos, de acordo com as características clinicas e histológicas: o *melanoma expansivo ou superficial* é um tipo de melanoma que apresenta desenvolvimento associado à exposição solar sazonal, com crescimento radial e posteriormente invasão tecidual. Trata-se do tipo mais frequente e surge geralmente na faixa etária entre 40 a 50 anos. Nas mulheres surge principalmente nos membros inferiores e nos homens na região do tronco (1, 7-9). O *melanoma nodular*, o segundo mais comum (15 a 30% dos casos), ocorre mais frequentemente nas quinta e sexta décadas de vida, principalmente em indivíduos do sexo masculino. Apresenta-se como uma lesão nodular, elevada, de cor castanha, negra ou azulada. São frequentes a ulceração e o sangramento; existe a variante amelanótica, com superfície critematosa com crescimento vertical e metástases precoces (1, 7, 8). O *melanoma lentiginoso acral* (MLA), surge nas regiões palmoplantares, extremidades digitais, mucosas e semimucosas; é mais frequente em indivíduos de pele negra (35 a 60%). Essa forma clínica não tem predileção por sexo e é mais frequente na sétima década

de vida. Nas extremidades digitais pode se apresentar como lesão tumoral acastanhada subungueal, melanoníquia estriada, fragmentação longitudinal da lâmina ungueal, além de paroníquia crônica e persistente. É o tipo histológico mais agressivo dentre os melanomas cutâneos (10, 11). O quarto tipo é o *melanoma lentigo maligno* (MLM), pouco frequente (apenas 5% dos casos); é mais comum em idosos; surge em área de fotoexposição crônica (11), apresenta-se como mancha acastanhada ou enegrecida, de limites nítidos e irregulares, alcançando vários centímetros de diâmetro, localizada na face (90%), em mãos e membros inferiores (10%) (11, 12).

1.2 Caquexia associada ao melanoma cutâneo

Uma síndrome que acometem muitos pacientes oncológicos conhecida como caquexia é definida por perda progressiva de massa muscular esquelética, na presença ou não de perda de tecido adiposo, que não pode ser revertida pelo suporte nutricional convencional (13,14). A caquexia é associada inicialmente com a ação normal do câncer, consequentemente ao crescimento tumoral e presença de metástases (13-15).

A manifestação da caquexia no melanoma cutâneo ocorre como nas demais variedades de canceres humanos (13-15). Uma caracterização dos aspectos da caquexia associado ao câncer foi desenvolvido no modelo experimental com uso de células de melanoma B16 inoculadas em camundongos C57BL/6 para o desenvolvimento tumoral, analisou que a gradação tumoral ocorreu paralelamente com a diminuição do tecido adiposo branco e redução da musculatura esquelética estriada dos animais (16). A molécula melanocortina é responsável pela ativação de neurônios hipotalâmicos com o objetivo de integrar sinais periféricos que controlam negativamente o apetite e a sede, por consequência do quadro inflamatório, parece estar descompensado, assim diminui o desempenho físico e transtorna a relação metabólica no organismo (17). Essa informação dialoga com o resultado provocado pela caquexia no melanoma cutâneo, configurando um papel importante na mediação da caquexia associada nessa doença (18). Outra situação intrigante é a inibição da lipoproteina lípase (LPL) oriunda de melanoma ou o fator inibitório de leucemia, essa condição desempenha uma ação importante na acentuação do quadro de caquexia em camundongos imunocomprometidos com melanoma induzido com células imortalizadas de melanoma B16 (16,18,19).

Reforçando a ocorrência do processo de caquexia no melanoma cutâneo, diversas linhagens de células primárias de melanoma humano são capazes de induzir uma intensa atividade lipolítica no tecido adiposo branco de indivíduos com esse problema, essa característica é fundamental para o surgimento e o desenvolvimento da caquexia nos pacientes com esse tipo de câncer (20,21).

1.3 Sistema ubiquitina-proteassoma: sistema de degradação proteolítico intracelular

Os organismos vivenciam constantemente um processo proteico intracelular e extracelular de síntese, degradação e substituição de nossas células e tecidos (22). Chama a atenção o fenômeno de proteólise (degradação proteica), realizada por sistemas proteolíticos intracelulares com ações complexas e estritamente coordenadas, objetivando a regulação de inúmeras classes de proteínas que tem diferentes períodos de vida e funções (23).

Os principais sistemas proteolíticos em células eucariontes são: o sistema ubiquitina-proteassoma, o sistema de cascata de caspases, o sistema de proteases lisossomais (catepsinas), as calpaínas cálcio-dependentes e o sistema autofágico (22-24). O sistema ubiquitina-proteassoma (SUP) é potencializado no processo de diversas doenças, entre elas, o câncer contribui significativamente para aumentar a ações do SUP, por consequência produz um prognóstico negativo no paciente acometido (18-24).

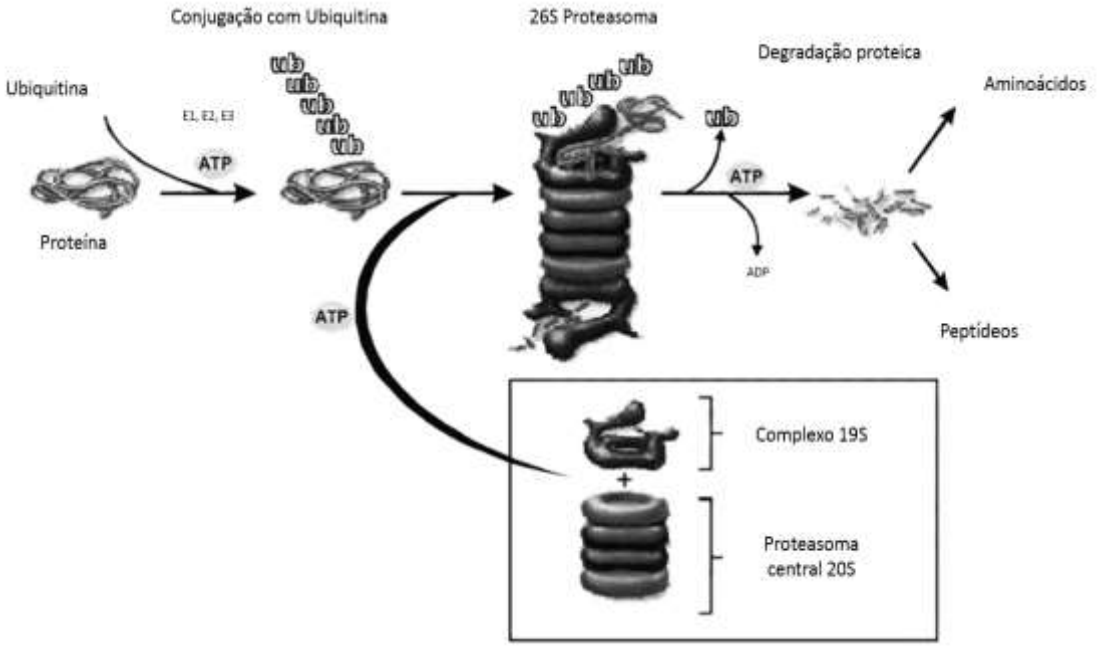
O SUP degrada a maioria das proteínas intracelulares, esse processo apresenta várias etapas de regulação, o que lhe confere uma notável precisão no reconhecimento de proteínas a serem degradadas (24-26). A degradação de proteínas pelo SUP se inicia a partir da ação coordenada de enzimas (ubiquitinas) que montam uma grande cadeia polipeptídica responsável pela identificação (juntamente com proteínas chaperonas) e transporte do substrato proteico para degradação (ubiquitinização) (22-26). O processo inicial envolve a interação da Ub com três componentes enzimáticos, a E1 (Ub-enzima ativadora), E2 (Ub-transportador da proteína Ub-conjugada) e a E3 (Ub-ligase) que reconhece a enzima E2 associada ao substrato proteico, sendo esse conjunto (E2-E3-substrato-5Ub) transportado até o proteassoma 26S (22-27). O proteassoma 26S é uma complexa protease multicatalítica composta por 60 subunidades (do tipo α e β) que se organizam na forma de um cilindro arranjado em quatro anéis polipeptídicos ocos empilhados (20S) com um orifício central por onde transita o substrato proteico a ser

degradado (24-28). Na face externa do cilindro se encontram as subunidades α ou subunidades estruturais (24-27). Na parte interna do cilindro há subunidades β, que contém os sítios ativos para a proteólise (com atividades hidrolíticas tipo quimiotripsina, tripsina, e pós-glutamil) (25-27). O proteassoma 20S está acoplado à partícula reguladora (19S) localizada em uma ou ambas de suas extremidades (20). Essas partículas reguladoras reconhecem o complexo Ub-E-substrato, liberando a entrada do substrato para dentro do proteassoma 20S para degradação catalítica e liberando a proteína Ub-conjugada (22-28). Após ação catalítica proteassomal, peptidases citoplasmáticas terminam a degradação de peptídeos em aminoácidos (25).

A montagem do proteassoma é um processo complexo devido ao número de subunidades que devem se associar para formar um complexo ativo. As subunidades β são sintetizadas como pró-peptídeos N-terminais que sofrem modificações pós-tradução durante a montagem do proteassoma 20S (24-27).

A partícula 20S é montada a partir de duas meia-proteassomas, cada uma consistindo de sete anéis pró-β ligados (24-27). A associação entre um anel β com as duas meia-proteassomas desencadeia a autólise treonina-dependente dos pró-peptídeos para expor os sítios ativos de clivagem (24-27). Essas interações β são mediadas principalmente por pontes salinas e interações hidrofóbicas entre as alfa-hélices conservadas (24-27). A montagem das meias-proteossomas, por sua vez, é iniciada pela montagem das subunidades α em seu anel heptamérico, formando um modelo para a associação do anel pró-β correspondente (24-27). No caso da partícula reguladora 19S, essa é montada como dois subcomponentes distintos: uma base e uma tampa (26-29). A montagem do complexo da base é realizada por proteínas chaperonas que se ligam às subunidades AAA-ATPase da partícula reguladora 19S (26-27). O complexo da tampa da partícula 19S é montada separadamente e não requer a participação de chaperonas (27,28). A montagem e a atividade funcional do proteassoma 26S podem ser reguladas pela alteração dos níveis de proteassoma, das proteínas reguladoras (ativadoras da partícula 20S do proteassoma, tais como: *PSME1, PSME2, PSME3, PSME4*; ativadoras da partícula 19S do proteassoma, tais como: *PSMD4, PSMD11* do proteassoma, ou de proteínas do sistema de conjugação da ubiquitina (ativadoras da ubiquitinação, tais como: *UBC, FBXO32 e TRIM-63*; inibidoras da ubiquitinação, tais como a *USP14* (26-27). Dessa forma, as células podem ajustar a sua capacidade de proteólise dependente do SUP por alterar as proteínas que participam na seleção, montagem e/ou na degradação do substrato proteico (24-27).

Alterações na expressão de componentes do proteassoma e outras proteínas reguladoras têm sido documentadas em um grande número de condições fisiológicas e patológicas, muitas delas associadas com a atrofia do músculo esquelético, como consequência do aumento das taxas de degradação global de proteínas (23-27). Na figura 1 pode ser visualizada a via ubiquitina-proteassoma.



Fonte: Adaptado de Mitch; Goldberg (1996)

Figura 1 - Via ubiquitina-proteassoma, é o principal sistema de catabolismo proteico, a degradação envolve a marcação da proteína e consequentemente a degradação da proteína marcada pelo proteassoma 26S.

1.4 Atividade física

A atividade física (AF) é definida por qualquer movimento corporal produzido pela musculatura que resulte num gasto de energia acima do nível de repouso, já o exercício físico é uma forma de atividade física planejada, repetitiva que visa desenvolvimento físico e as habilidades motoras (28,29).

A atividade física é uma promotora na redução das taxas de mortalidade (28,29). Pois a inatividade física e o baixo nível de condicionamento físico são considerados fatores de risco

para diversas doenças como a hipertensão arterial, a resistência à insulina, o diabetes, a dislipidemia, a obesidade entre outras. Assim a pratica regular e/ou o aumento da atividades físicas diárias é recomenda para prevenção e tratamento de diversas situações patológicas (28-30).

A tecnologia contribuiu para o homem adotar um estilo de vida mais sedentário, os apontamentos epidemiológicos demostram que hábitos saudáveis que incluam a atividade física diária, tem impacto positivo sobre a aptidão física relacionada a saúde (31).

Pensando no conceito de AF podemos definir a *Atividade Física Espontânea* (AFE) como qualquer movimento involuntário que não contenha um controle severo, havendo a possibilidade de ser executado a qualquer momento. Esse conceito ganhou força em função de estratégias metodológicas para modelos de atividade física para roedores, além disso, possibilita a junção da ATE com o ambiente enriquecido (AE) ou não (32). O AE se caracteriza pela composição de instrumentos'' lúdicos'', com objetos coloridos, de diferentes formas, tamanho e textura; rodas; alguns brinquedos e abrigos (33). Esse conjunto de objetos tem como objetivo fornecer aos animais movimentos naturais da espécie. Esses movimentos geram efeitos comportamentais e morfológicos (33).

1.5 Exercício resistido

O exercício resistido é conhecido como exercício de força, exercício contra resistência ou musculação, o processo longitudinal não caracteriza como exercício, mas sim, como treinamento (34-39). A atividade dessa modalidade de exercício é uma prática popular entre atletas e não atletas em geral (37-39).

O exercício resistido é caraterizado pela utilização de máquinas ou pesos livres para oferecer um carga mecânica oposta ao movimento do segmento corporal, provocando assim adaptações neurais e hipertróficas no indivíduo (36-39).

As adaptações biológicas promovidas pelo exercício resistido apresentam um reconhecido na prevenção e promoção da saúde em geral (38-40). Uma das importantes modificações promovidas pelo treinamento resistido é a manutenção e/ou aumento do tecido muscular estriado esquelético (36-40). O tecido muscular estriado esquelético tem a capacidade de se

adaptar aos diversos estímulos do treinamento resistido (37-40). No exercício resistido, o exercício pode promover um acréscimo substancial da área de secção transversa do músculo e melhorar as respostas funcionais de força muscular e potência, além de sintetizar diversos hormônios, citocinas anti-inflamatórias e fatores tróficos, o que influência positivamente o funcionamento sistêmico (41-44). Tal resposta anabólica ocorre predominantemente pela via de integração proteica de fator de crescimento semelhante à insulina tipo 1/PI3K/Akt/mTOR (IGF1/PI3K/Akt/mTOR), que apresenta elevada expressão após a finalização do exercício resistido (41-44). O exercício resistido proporciona efeitos antagônicos ao da ativação do SUP, pois enquanto este aumenta o catabolismo proteico, o exercício resistido estimula o aumento da hipertrofia muscular (42-45).

Após o exercício resistido ocorre a ativação das proteínas de cascatas sinalizadoras que regulam os processos de transcrição e tradução, consequentemente aumentando a síntese de proteínas (45-49). Assim é capaz de promover maior ativação de Akt aguda e cronicamente (48-51). É importante salientar que além do aumento da ativação de mTOR, promovida inicialmente pela produção de IGF-1 e conduzindo uma cascata de ativação por PI3K, *pyruvate dehydrogenase kinase* 1 e 2 (PDK1 e PDK2) e Akt, ocorre também a inibição da enzima *glycogen sintase kinase beta* (GSK-3β), reduzindo a inibição da síntese proteica e contribuindo para a hipertrofia muscular esquelética (42-50). Em adição, o remodelamento da musculatura esquelética também é promovido pela via calcineurina/NFAT e pelas células satélites (50-51).

A via calcineurina/NFAT está envolvida no mecanismo de transição/modulação das fibras musculares estriadas esqueléticas que é regulada de acordo com estímulos extrínsecos (carga mecânica, estimulação elétrica) e/ou intrínsecos (níveis intracelulares de glicogênio, ATP, K^{+}, Ca^{+2}, O_2) (49-53). Elevados concentrações de íons de Ca^{+2} durante o processo de contração muscular ocorre o estimulo para ativação da calcineurina, que posteriormente ativa a transcrição gênica e remodelamento muscular. Portanto, essa via está relacionada à alteração do perfil fenotípico das fibras musculares estriadas esqueléticas de acordo com o direcionamento de intensidade e volume do treinamento resistido (48-52). Por outro lado, as células satélites são encontradas entre a lâmina basal e o sarcolema do miócito esquelético e tem a capacidade de se especializar e fundir-se, estruturando novas fibras musculares (46-52). A diferenciação ocorrida propicia a hipertrofia muscular após a danificação em função de fatores microlesivos, como por exemplo, pelo exercício resistido (46-52), que é iniciador da resposta inflamatória aguda, a qual proporciona um aumento na migração de macrófagos e neutrófilos para a estrutura

muscular. Tal resposta é importante para ativar a ação das células satélites por meio da IL-6, promovendo um ambiente anabólico após a sessão do exercício de força (45-52).

Outra situação importante é o estímulo da Akt promovido pela deformação mecânica das fibras musculares na sessão de exercício resistido, que promove a fosforilação do fator de transcrição *fork-head Box o family* (FOXO), inibindo o incitamento da transcrição de ubiquitinas ligases do SUP (45-52). A sobrecarga do exercício resistido resultaria no decréscimo da ação do SUP, pois a inibição da FOXO reduz a expressão de genes das proteínas ubiquitinas ligases atrogin-1, *muscle RING finger 1* (MuRF1) e *muscle atrophy F-box* (MAFbx), reduzindo a ubiquitinização e, consequentemente, o catabolismo muscular (48-52). Portanto, a hipertrofia muscular esquelética induzida pelo exercício resistido pode ocorrer devido à ativação de IGF-1/PI3K/AKt/mTOR e inibição de FOXO (48-52).

1.6 O potencial do exercício físico na caquexia associada ao câncer

As adaptações provocadas pelo exercício físico apresentam um impacto na prevenção de diversas doenças, dentre elas o câncer (29-39). Um ajuste importante promovido pelo exercício físico é a manutenção e/ou aumento do tecido muscular estriado esquelético, esse tecido corresponde cerca de 40% a 50% do peso corporal total, assim a sua diminuição abrupta contribui negativamente com a sobrevida (29-39).

Essa estrutura muscular tem a capacidade de adaptar as diversas respostas do treinamento físico (29-38). No exercício resistido, pode ocorrer um acréscimo substancial da área transversa do músculo, além disso, melhora os níveis de força muscular e potência (52), diferentemente do treino aeróbio, que promove a ação metabólica prioritariamente oxidativa, assim ocorre o aumento do número de mitocôndrias (54).

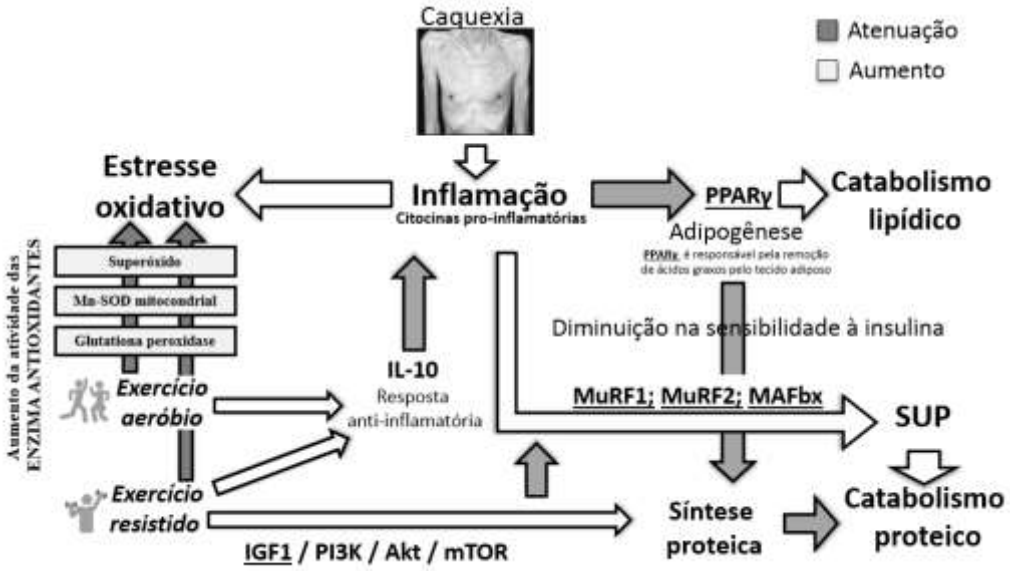
O exercício resistido também promove outras alterações além das descritas anteriormente, o que sugerem melhoras no quadro de caquexia geral (38, 52-54). No exercício resistido as respostas positivas na diminuição da inflamação crônica, é provocado pela síntese proteica aumentando a área de secção transversa das fibras musculares (53-55) O mecanismo que provoca a situação descrita anteriormente é estimulada predominantemente pela via IGF1/PI3K/Akt/mTOR onde foi observada em diversos estudos níveis elevados dos componentes dessa via após a finalização do exercício resistido (43-51).

O exercício físico em geral contribui para todas as vertentes que envolvem o câncer, estudos fornecem evidências que a prática do exercício físico promove diminuição na taxa de mortalidade (57). Em uma investigação com treinamento resistido associado à suplementação com óleo de peixe em ratos portadores do tumor de Walker 256, sobre os parâmetros da caquexia e crescimento tumoral, observou que o tratamento proposto foi eficaz em evitar a caquexia e induzir a redução do crescimento tumoral (58). Apesar da relação indireta do treino aeróbio, em um estudo que foi realizado 4 dias de atividade aeróbia voluntaria após inoculação de células tumorais C26 na estrutura muscular do tibial anterior de camundongos suíços, mostrou que o crescimento tumoral foi menor quando comparados com o grupo controle e que além disso apresentavam maior massa muscular esquelética (59). Outra situação importante é o aumento da sensibilidade à insulina e melhora da função endotelial, associada ao aumento da capacidade física e à redução da fadiga, promovida pelo exercício aeróbico em pacientes com câncer (54). Além disso, o aumento na síntese de citocinas antiinflamatórias (por exemplo, IL-10) e, consequentemente, a elevação da expressão de antagonistas de citocinas pró-inflamatórias (por exemplo, IL-1ra), contribuem para a redução da inflamação crônica, um ambiente ideal para a manutenção e/ou aumento do volume do tecido muscular (52,54).

O potencial do treino resistido é mostrado que em um estudo in vitro, onde a via IGF1 / PI3K / Akt pode bloquear a regulação positiva da ubiquitina ligase induzida pela ação da dexametasona na ativação de MuRF1 e MAFbx, promovendo um bloqueio da FOXO. Estudos mostram que a sinalização para hipertrofia muscular inibi a ação da FOXO (59, 60, 61), permitindo especular a possível inibição da SUP. Corroborando com o descrito acima, um estudo no qual os miotubos cultivados sofreram atrofia mostrou que a atividade da via PI3K/AKT diminui quando ocorre a ativação dos fatores de transcrição FOXO e a indução de atrogina-1. No entanto, o tratamento com IGF-1 ou superexpressão de Akt inibiu a expressão de FOXO e atrogina-1 (59- 62), o que reforça a relação antagônica dessas vias. É importante ressaltar que, para a regulação da integridade muscular, tanto o exercício aeróbico quanto o exercício resistido são fundamentais. Entretanto, efeitos com relação direta na célula muscular são observados em resposta ao treinamento resistido, uma vez que, além de diminuir a inflamação crônica, promove a síntese protéica responsável pelo aumento da área de secção transversa das fibras musculares (43,45). Tais mecanismos diretos e indiretos de inibição da SUP, além dos efeitos antioxidantes, anti-inflamatórios e anabólicos do exercício físico, criam um ambiente favorável à potencialização

do tratamento para pacientes com caquexia associada ao câncer, situação está amplificada por diversos trabalhos.

Em geral, o exercício físico pode reduzir a incidência de cânceres atribuídas ao sedentarismo (63), fato importante na prevenção desse problema de saúde pública enfrentado no Brasil e no Mundo (54-63), assim, a proposta não farmacológica de tratamento durante o prognostico da doença aparece como potencial positivo na intervenção clínica (48). Na figura 2 apresenta as possíveis situações que o exercício físico pode promover no organismo acometido pela caquexia associada ao câncer (52-62), destacando o exercício resistido, pois promove ações diretas no processo de síntese proteica e diminuição do catabolismo proteico através da atenuação da SUP. Já o exercício aeróbico contribui com a redução das espécies reativas de oxigênio, contribuindo para diminuir a ação do sistema ubiquitina-proteassoma, lembrando que o exercício resistido também contribui para tal situação (52-62).



Fonte: Adaptado de Gould, *et al* (2013)

Figura 2 - Possíveis mecanismos-chave ocorridos na caquexia associada ao câncer e a influência do exercício físico. O exercício aeróbico contribui com a redução das espécies reativas de oxigênio, contribuindo para diminuir a ação do sistema ubiquitina-proteassoma. O exercício resistido promove aumento da síntese protéica, atuando diretamente na inibição do processo catabólico.

2 OBJETIVOS

2.1 Objetivo geral

Investigar os efeitos do treinamento resistido e da atividade física na progressão tumoral e na ocorrência de caquexia associada ao modelo tumoral singênico murino de melanoma cutâneo em camundongos C57BL/6.

2.2 Objetivos específicos

- Elaborar um modelo de utilidade para exercício resistido com camundongos C57BL/6;

- Analisar os efeitos do exercício resistido utilizando a escalada com estimulo de choque elétrico nos níveis de força muscular, na composição corporal, no volume de membro, na área das fibras musculares esqueléticas e dos níveis plasmáticos de lactato em camundongos C57BL/6;

- Verificar os efeitos do exercício resistido e da atividade física na estrutura músculo esquelético, no tecido adiposo branco e na sobrevida de camundongos C57Bl/6 com caquexia cutânea relacionada ao melanoma.

3 PRODUTOS CIENTÍFICOS GERADOS

3.1 Modelo de utilidade:

Disposições introduzidas em escada para treinamento neuromuscular resistido de roedores

Local depositado: Instituto nacional de propriedade industrial.

3.2 Artigo Científico 1:

Methodological validation of a vertical ladder with low intensity shock stimulus for resistance training in C57BL/6 mice: effects on muscle mass and strength, body composition, and lactate plasma levels

Trabalho submetido e aprovado no periódico: Journal of Human Sport and Exercise

3.3 Artigo Científico 2:

Effects of resistance training and physical activity on skeletal muscle and white adipose tissues, myokines and adipokines gene expression, cancer-related cachexia occurrence, and cancer-related survival of C57BL/6 mice bearing syngeneic cutaneous melanoma

Periódico alvo: Jornal of Cachexia, Sarcopenia and Muscle

3.1 Modelo de utilidade:

Disposições introduzidas em escada para treinamento neuromuscular resistido de roedores

Local depositado: Instituto nacional de propriedade industrial.

PATENTE DE MODELO DE UTILIDADE:

Disposições introduzidas em escada para treinamento neuromuscular resistido de roedores

INVENTORES:

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RESUMO: A presente patente de invenção diz respeito a um instrumento de uso em pesquisas científicas, em ambientes de biotério, para possibilitar a investigação dos efeitos agudos e crônicos advindos do exercício resistido em roedores das linhagens: Swiss (Ordem Rodentia; Família Muridae; Gênero *Mus*; Espécie *Mus musculus*; Raça *Swiss*), camundongos C57Bl/6 (Ordem Rodentia; Família Muridae; Gênero *Mus*; Espécie *Mus musculus*; Raça *C57Bl/6*), camundongos Balb/c (Ordem Rodentia; Família Muridae; Gênero *Mus*; Espécie *Mus musculus*; Raça *Balb/c*) e ratos Wistar (Ordem Rodentia; Familia Muridae; Gênero *Mus*; Espécie *Mus musculus*; Raça *Wistar*) de forma similar ao que ocorre em seres humanos (Ordem Primates; Subordem Haplorrhini*;* Infraordem Simiiformes; Superfamilia Hominoidea; Família Hominidae; Subfamília Homininae; Tribo Hominini; Subtribo Hominina; Género *Homo*; Espécie *Homo sapiens*; Raça *H.s.sapiens*) quando submetidos ao exercício resistido (sinônimos: exercício contra resistência, exercício de força e musculação) específico. Este instrumento é caracterizado como uma escada vertical (1,1 x 0,18 m; degrau de 2 cm; inclinação de 80º) contendo um estimulador elétrico na base (área de saída) e uma câmara de descanso no topo da escada, onde está localizada a câmara de alimentação. Sua utilização permitirá avançar na análise de diversas interações fisiológicas e moleculares que envolvem o exercício resistido em um organismo.

RELATÓRIO DESCRITIVO DE PATENTE DE MODELO DE UTILIDADE

Campo da Invenção

[001] A presente patente de invenção diz respeito a um instrumento de uso em pesquisas científicas, em ambientes de biotério, para possibilitar a investigação dos efeitos agudos e

crônicos advindos do exercício neuromuscular resistido em roedores das linhagens Swiss (Ordem Rodentia; Família Muridae; Gênero *Mus*; Espécie *Mus musculus*; Raça *Swiss*), camundongos C57Bl/6 (Ordem Rodentia; Família Muridae; Gênero *Mus*; Espécie *Mus musculus*; Raça *C57Bl/6*), camundongos Balb/c (Ordem Rodentia; Família Muridae; Gênero *Mus*; Espécie *Mus musculus*; Raça *Balb/c*) e ratos Wistar (Ordem Rodentia; Familia Muridae; Gênero *Mus*; Espécie *Mus musculus*; Raça *Wistar*) de forma similar ao que ocorre em seres humanos (Ordem Primates; Subordem Haplorrhini*;* Infraordem Simiiformes; Superfamilia Hominoidea; Família Hominidae; Subfamília Homininae; Tribo Hominini; Subtribo Hominina; Género *Homo*; Espécie *Homo sapiens*; Raça *H.s.sapiens*). É caracterizado como uma escada vertical (1,1 x 0,18 m; degrau de 2 cm; inclinação de 80º) contendo um estimulador elétrico na base (área de saída) e uma câmara de descanso no topo da escada, onde está localizada a câmara de alimentação. É direcionado, especialmente, a empresas privadas que investem na criação de produtos de interesse à pesquisa experimental animal, educadores e pesquisadores de Instituições de Ensino Superior e Centros de Pesquisas cujos projetos de ensino e pesquisa pretendem avaliar efeitos que ocorrem na musculatura esquelética a partir do exercício resistido que advém com o uso do presente instrumento.

Fundamentos da Invenção

[002] A escada vertical com estimulador elétrico é um instrumento que apresenta a possibilidade de mudança no formato dos treinos neuromusculares para seres vivos pertencentes à família Muridae, de modo a proporcionar ganho de força muscular, com consequente hipertrofia e aumento de resistência muscular.

[003] O treino resistido (sinônimos: treino de força, treino contra resistência ou musculação) convencional descrito pelo ato de subir uma escada vertical por meio de quatro apoios exige utilização de diversas capacidades físicas, dentre elas, se destaca a força muscular dos membros.

[004] Há um formato de exercício resistido que utiliza o modelo de agachamento proposto por Tamaki e colaboradores (*Tamaki T, Uchiyama S, Nakano S. A weight-lifting exercise model for inducing hypertrophy in the hindlimb muscles of rats. Medicine and science in sports and exercise, 1992; 24;8, 881-886*) para a realização do treinamento de força, projetado de maneira que o animal fique imobilizado sobre uma plataforma metálica por meio de um colete adaptado acoplado ao tórax. O processo é feito com animais conectados a uma barra de madeira móvel de 35 cm, na qual são alocadas as anilhas de sobrecarga. Os animais acoplados à jaqueta permanecem em posição sentada com suas patas traseiras flexionadas e apoiadas na base de

sustentação. Para que o roedor realize o salto (movimento de flexo-extensão completa de joelho e tornozelo) erguendo uma carga posicionada na parte posterior do colete, aplica-se sobre ele uma estimulação elétrica por meio de um clipe metálico, que envolve a extremidade da cauda do animal, ligado a um eletroestimulador. Em seguida, utilizam um estimulador elétrico (modelo Dualpex 961, Quarker) para induzir a atividade física por meio de eletrodos autoadesivos (modelo CFE200 e tamanho 3,2 cm) posicionados na cauda das espécimes. Como resultado da eletroestimulação, os animais realizam, repetidamente, a extensão dos joelhos, elevando a sobrecarga fixada ao aparato.

[005] O referido estado da técnica inviabiliza as pesquisas neuromusculares, porque o animal não tem a estimulação de todos os membros, mas somente das patas traseiras e a relação de intensidade e volume de treino não têm formas mensuráveis. Normalmente é aplicado em exercícios tradicionais, como musculatura das pernas (*leg press*), extensão de joelhos e flexão de joelhos.

[006] Outro formato utilizado de treino resistido foi descrito por Homerberg e Farrar (*Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance exercise in the rat. Can Journal Appl Physiol*, 29 1: 16-31, 2004) citado por diversos pesquisadores (RODRIGUES, Marcella Damas; BORIN, Sergio Henrique; DA SILVA, Carlos Alberto). Relações metabólicas em ratos sob o treinamento anaeróbio em escada (Revista Brasileira de Ciências do Esporte, v. 39, n. 1, p. 63-67, 2017), os quais descrevem o protocolo que consiste em subida de escada (escalada) (1,1x 0,18, 2cm de espaçamento entre os degraus da grade, com inclinação de 80º), utilizado para treinamento anaeróbico (força), no qual o animal é estimulado manualmente e deve ser muito treinado (domesticado com a escalada) antes de iniciar a sequência de exercícios. No estado da técnica apresentado, o animal não é elevado à possibilidade de recrutamento total de unidades motoras da estrutura muscular esquelética, pois a estimulação manual ou voluntária do animal não proporciona eficiência na proposta de exercício resistido similar em seres humanos. O treino é ainda prejudicado pela constante paralisação das atividades, em função da grande incidência de fugas dos animais pelas laterais e na base da escada, por ser desprovida de contenções.

[007] O uso dessas metodologias descritas para o exercício resistido não atendem as demandas de treinos neuromusculares, visto que não proporcionam ganho de força muscular, com consequente hipertrofia e aumento de resistência muscular. Na tentativa de aperfeiçoar a técnica, foram introduzidas disposições à escada convencional utilizada para treino resistido, de modo a promover a movimentação vertical do animal no sentido base-ápice, minimizando a intervenção manual do pesquisador, bem como a fuga dos animais, para proporcionar trabalho

muscular esquelético resistido, de modo a possibilitar adaptações neurais (coordenação intramuscular e intermuscular, desenvolvimento da sincronização e aumento de recrutamento de unidades motoras) e hipertróficas (hipertrofia sarcoplasmática e hipertrofia miofibrilar) para camundongos e ratos.

[008] No estado da técnica atual não são apresentados equipamentos que possibilitem movimentação contínua e com a máxima solicitação de membros dianteiros e traseiros no sentido vertical, objetivando os treinos para pesquisas neuromusculares, que demandam ganho de força muscular, hipertrofia muscular, força explosiva ou resistência muscular localizada.

Sumário da Invenção

[009] As disposições introduzidas na escada, a exemplo da câmara de estimulação elétrica e contenções laterais, proporcionam trabalho neuromuscular com adaptações agudas e crônicas em maior quantidade do tecido muscular estriado esquelético, visando o aumento da força muscular e suas variações (formas de manifestação) em camundongos e ratos.

[010] Trata-se se de uma ferramenta que pode ser amplamente utilizada por pesquisadores, passível de escalabilidade industrial, possui baixo custo, grande durabilidade, baixa incidência de manutenção e é de simples manuseio.

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[014] Figura 4 - Representa o dispositivo numa visão posterior;

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[017] Figura 1 no item 2 – Câmara de alimentação (Estimulador olfativo);

[018] Figura 1 no item 3 – Câmara de descanso;

[019] Figura 1 no item 4 – Coluna de sustentação;

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[051] Figura 5 no item 13 – Painel de estimulação elétrica.

Descrição Detalhada da Invenção

[052] As disposições introduzidas para constituição da patente de modelo de utilidade para treinamento neuromuscular resistido de roedores é um instrumento que apresenta-se na forma de uma escada vertical com um estimulador elétrico acoplado (item 6 na figura 1) contém um painel de estimulação elétrica (item 13 da figura 1) de 150 milímetros de altura, 288 milímetros de comprimento (com inclinação de 30º), com 190 milímetros de largura, com um interruptor redondo de 20 milímetros de diâmetro e 27,5 milímetros de profundidade de ligar e desligar o estimulador elétrico, com dois botões interruptores de 10 milímetros de diâmetro e 15 milímetros de comprimento para intensidade e frequência respectivamente, com um circuito inversor DC\AC, com base do circuito integrado 555, onde pode gerar tensões AC de até 60 Volts Esse estimulador está posicionado na base horizontal da escada (item 5 da figura 1), onde se localiza um uma câmara de estimulação elétrica (item 8 na figura 1) que possui, na parte superior, uma grade de contenção da câmara de estimulação elétrica (item 7 da figura 1) e contenções laterais (item 9 da figura 1) totalmente fechadas, verticalizadas em inclinação de 80º, com o intuito de dificultar a fuga dos espécimes da escada durante o treino resistido.

[053] No decorrer da subida, com cerca de 1,1m, há uma escada com 43 degraus (item 11 da figura 1) e, abaixo destes degraus, há uma base vertical (item 10 na figura 1).

[054] No topo da estrutura da escada de treino resistido há uma câmara de descanso (item 3 da figura 1) onde, no topo da escada, dentro dessa câmara há uma grade de contensão (item 1 da figura 1) que separa uma área específica com o propósito de estimulação olfativa. Essa área é chamada de câmara de alimentação (item 2 da figura 1).

REIVINDICAÇÕES

1. DISPOSIÇÕES INTRODUZIDAS EM ESCADA PARA TREINAMENTO NEUROMUSCULAR RESISTIDO DE ROEDORES É CARACTERIZADA COMO um instrumento no formato de uma escada vertical com uma grade de contenção (Figura 1 no item 1), Câmara de alimentação (Figura 1 no item 2), Câmara de descanso (Figura 1 no item 3), Coluna de sustentação (Figura 1 no item 4), Base horizontal da escada (Figura 1 no item 5), Estimulador elétrico (Figura 1 no item 6), Grade de contenção da câmara de estimulação elétrica (Figura 1 no item 7), Câmara de estimulação elétrica (Figura 1 no item 8), Contenção lateral (Figura 1 no item 9), base vertical (item 10 da figura 1), com 43 degraus (item 11 da figura 1), Passagem entre a escada e a câmara de descanso (item 12 da figura 1) e Painel de estimulação elétrica (item 13 da figura 1).

2. DISPOSIÇÕES INTRODUZIDAS EM ESCADA PARA TREINAMENTO NEUROMUSCULAR RESISTIDO DE ROEDORES, de acordo com a reivindicação 1, **caracterizada por** compreender no formato de escada vertical com 1129 milímetros de altura da escada, 1348 milímetros totais de altura do equipamento, com a base de 300 milímetros de largura, com 43 degraus de largura de 0,02 milímetros e com inclinação de 80º.

3. DISPOSIÇÕES INTRODUZIDAS EM ESCADA PARA TREINAMENTO NEUROMUSCULAR RESISTIDO DE ROEDORES, de acordo com a reivindicação 1, **caracterizada por** com um estimulador elétrico (item 6 na figura 1), contém um painel de 150 milímetros de altura, 288 milímetros de comprimento (com inclinação de 30º), com 190 milímetros de largura, com um interruptor redondo de 20 milímetros de diâmetro e 27,5 milímetros de profundidade de ligar e desligar o estimulador elétrico, com dois botões interruptor de 10 milímetros de diâmetro e 15 milímetros de comprimento para intensidade e frequência respectivamente, com um circuito inversor DC\AC, com base do circuito integrado 555, onde pode gerar tensões AC de 60 Volts.

4. DISPOSIÇÕES INTRODUZIDAS EM ESCADA PARA TREINAMENTO NEUROMUSCULAR RESISTIDO DE ROEDORES, de acordo com a reivindicação 1, **caracterizada por** base (item 5 da figura 1) onde localiza um uma câmara de estimulação elétrica (item 8 na figura 1), contém 210 milímetros de altura, sendo 60 milímetros de grade na parte superior, contém 290 milímetros de comprimento, contém 200 milímetros de largura, local de alojamento do estimulação elétrica (item 8 na figura 1).

5. DISPOSIÇÕES INTRODUZIDAS EM ESCADA PARA TREINAMENTO NEUROMUSCULAR RESISTIDO DE ROEDORES, de acordo com a reivindicação 1, **caracterizada por** corrimões laterais (item 9 da figura 1, item 3 da figura 2 e item 4 da figura 5) caracterizado por 125 milímetros de altura, por 1129 milímetros de comprimento.

6. DISPOSIÇÕES INTRODUZIDAS EM ESCADA PARA TREINAMENTO NEUROMUSCULAR RESISTIDO DE ROEDORES, de acordo com a reivindicação 1, **caracterizada por** câmara de descanso (item 3 da figura 1, item 7 da figura 2, item 3 da figura 3, item 4 da figura 4 e item 5 da figura 5) com 219 milímetros de altura, 200 milímetros de comprimento, por 295 milímetros de largura.

7. DISPOSIÇÕES INTRODUZIDAS EM ESCADA PARA TREINAMENTO NEUROMUSCULAR RESISTIDO DE ROEDORES, de acordo com a reivindicação 1, **caracterizada por** grade de contensão (item 1 da figura 1) que separa um área específica para a proposta de estimulação olfativa, área chamada de câmara interna de alimento (item 2 da figura 1) com 217 milímetros de altura, por 293 milímetros de largura.

FIGURAS DO PROTÓTIPO

Figura 1

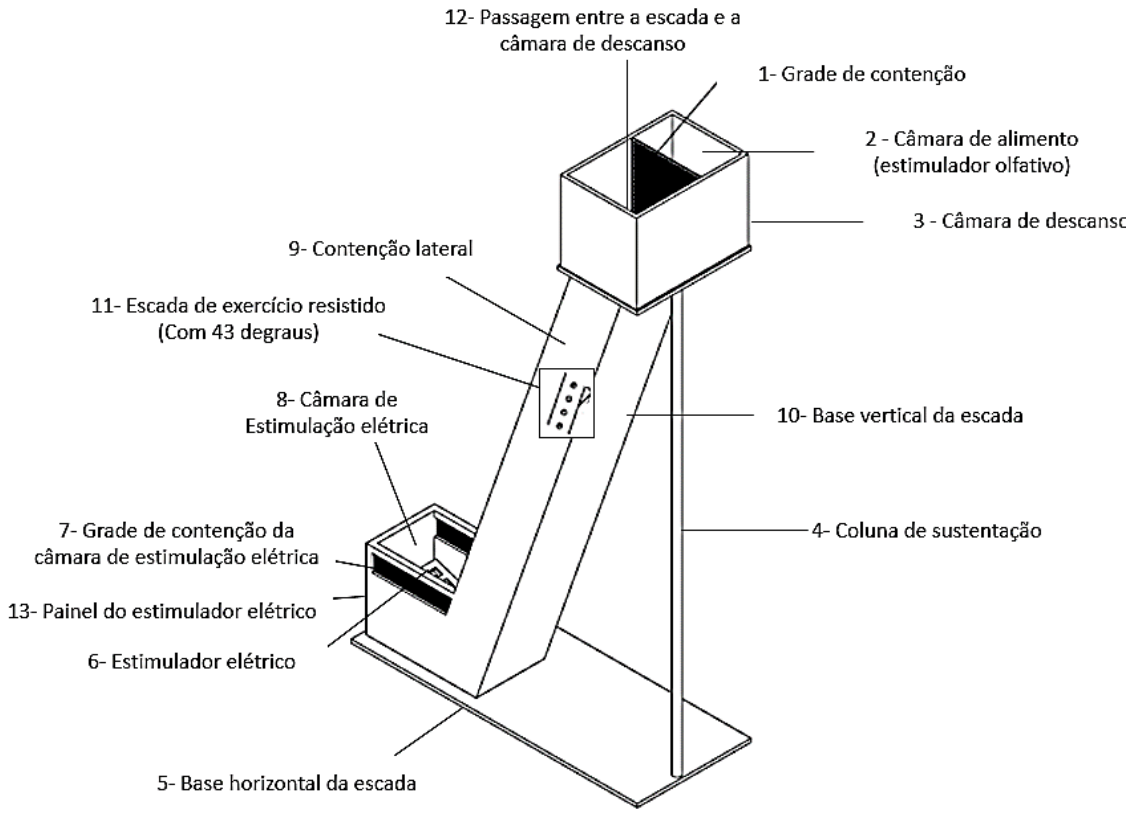


Figura 2

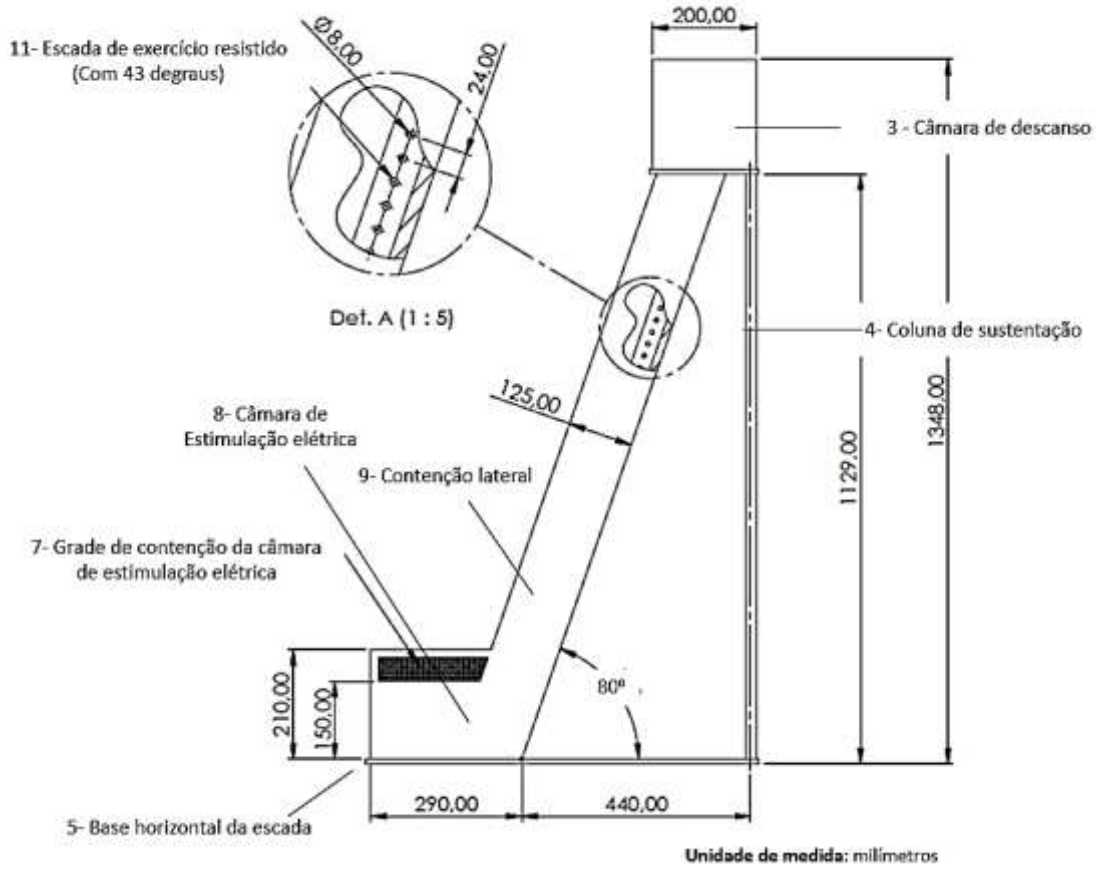


Figura 3

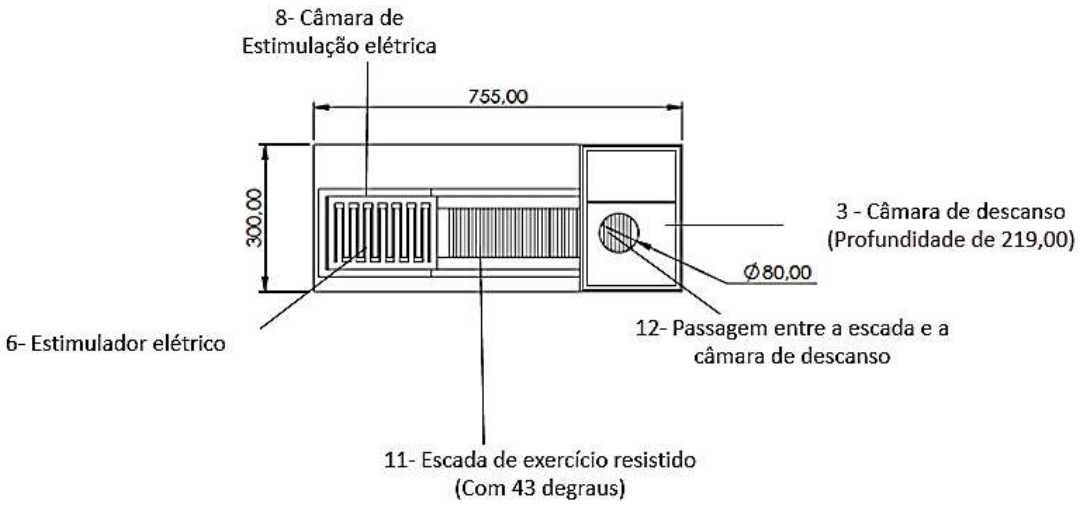


Figura 4

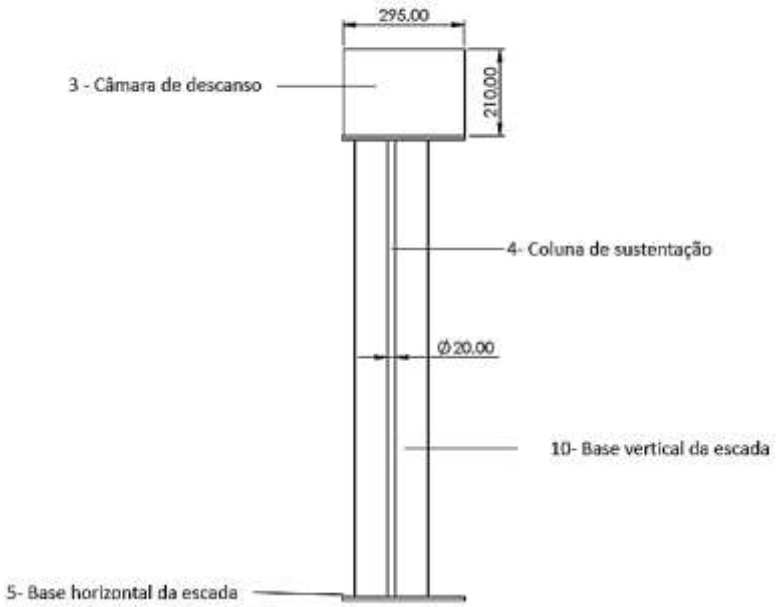
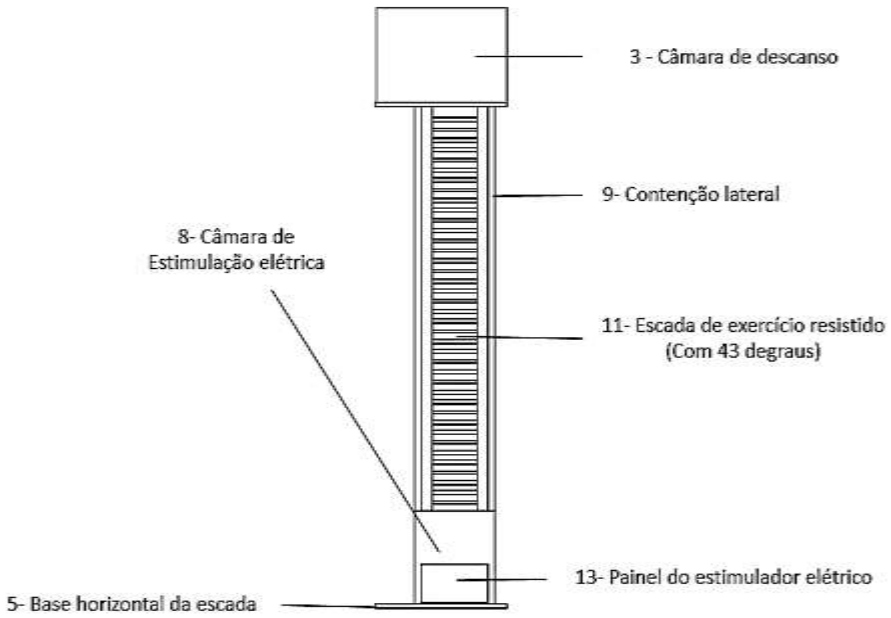


Figura 5



3.2 Artigo Científico 1:

Methodological validation of a vertical ladder with low intensity shock stimulus for resistance training in C57BL/6 mice: effects on muscle mass and strength, body composition, and lactate plasma levels

Trabalho submetido e aprovado no periódico: Journal of Human Sport and Exercise

Methodological validation of a vertical ladder with low intensity shock stimulus for resistance training in C57BL/6 mice: effects on muscle mass and strength, body composition, and lactate plasma levels

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ABSTRACT

BACKGROUND: The objective of this study was to evaluate the effects of a vertical ladder device for resistance exercises with or without electrical shock stimulus on muscle strength, body composition, limb volume, muscle fibers and plasma lactate of female mice. This device is represented by a vertical ladder with electrostimulation. It was analyzed in groups of C57BL/6 mice practicing spontaneous physical activity in enriched environment, practicing resisted climbing exercises, practicing resistance exercises with the utility model in question and controls. The acute effects of blood lactate and dark light-box behavior, and the short-term chronic effects of muscle strength, limb volume, body composition, muscle fiber area, and central and light-dark quantification were verified. According to the findings, the vertical electrostimulation ladder model presented acute effects on lactate levels, similar to other experimental models of resistance exercise and physical activity. The behavior in the light-dark box test showed no difference between the groups. Regarding the short-term chronic response, the best results were obtained in the impact-stimulated resistive exercise in the limb traction muscle variables, greater brown adipose tissue weight, greater quadriceps femoral muscle structure, limb and greater weight number of nuclei in the skeletal striated muscle fibers. The use of the prototype showed similarities in the acute and chronic adaptations expected in resistance training. However, new study proposals should be encouraged, as the data presented here are the first notes on the use of this utility model.

Keywords: Physical exercise; Strength training; Ladder exercise with electrostimulation; Validation.

INTRODUCTION

Resistance exercise, also known as strength exercise, is characterized by the use of equipment or free weights that offer a mechanical load opposite to the movement of the body segment, provoking neural and/or hypertrophic muscular adaptations in the individual (Haff & Triplett, 2015; Kenney, Wilmore, & Costill, 2015; Magbanua et al., 2014; Medicine, 2002; Schnyder & Handschin, 2015). The resistance exercise programs aimed at enhancing the trainable physiological characteristics such as muscular strength, muscular hypertrophy, muscular power and muscular endurance (Haff & Triplett, 2015; Hornberger Jr & Farrar, 2004). The maintenance time of the tension, the speed of execution of the movement, the type of accumulative metabolic, among other situations, are possibilities of the variability of stimuli that promote structural and functional adaptations in the corporal system (Haff & Triplett, 2015; Hornberger Jr & Farrar, 2004; Medicine, 2002).

The improvement of muscle strength primarily uses the anaerobic non lactic and/or lactic energy system (Haff & Triplett, 2015). In this energetic system, neural and hypertrophic muscular adaptations occur mainly in skeletal muscle fibers of fast contraction (fiber type II) recruited (Medicine, 2002; Ratamess, Alvar, & Evetoch, 2009). Skeletal muscle fibers of slow contraction (type I fiber) are most used in aerobic exercises that primarily use oxidative metabolism (Soltow et al., 2006). Neural adaptations are characterized by increased muscle strength, improved intermuscular and intramuscular coordination (Medicine, 2002; Spiering et al., 2008). The muscular hypertrophic adaptations are structural or morphological adaptations that occur due to the increase in the density and volume of muscle fibers (myofibrillar hypertrophy) or the increase of structures and components that will increase the efficiency of muscle fibers contraction (sarcoplasmic hypertrophy) (Hornberger Jr & Farrar, 2004; Spiering et al., 2008).

In rodents, resistance training methodologies include climbing (11), isometric training (Krüger et al., 2013), voluntary climbing (Mori et al., 2003), burrowing (Roemers et al., 2017) and shock-stimulated jumping exercises (Nuzzo, 2014), and the results achieved are quite similar to those that are demonstrated in a training study in humans. However, the literature presents some shortcomings related to exercise efficiency in mimicking the findings that occur in the human model; the prescription of protocol; and the apparatuses that can be used in the experimental models to achieve even more similar results with what occurs in humans.

Thus, in an attempt to promote greater efficiency in the physiological results provoked by experimental resistance exercise, a device for resistance training was developed that represented by a suitable vertical ladder with electric shock stimulus. Such a device possibly causes extensive muscle recruitment of the four rodent members. In the present study, the objective of this study was to evaluate the effects of a vertical ladder device for resistance

exercise with or without electric shock stimulation on a muscle strength, body composition, limb volume, muscle fibers and plasma lactate levels of female mouse.

MATERIAL AND METHODS

Type of study and ethical aspects

The present validation study is an experimental, analytical, prospective and quantitative approach. It was submitted for analysis by an ethics committee on animal experimentation and welfare and received a favorable opinion for its execution (CEEBEA/Unimontes, Process number: 131/2017).

Vertical ladder apparatus

The equipment that was submitted to methodological validation has a design similar to that of a typical vertical ladder to perform resistance exercises for rats (Cassilhas et al., 2012; Cassilhas et al., 2013). This apparatus contain grid, feeding chamber, resting chamber, support column, horizontal ladder base, electric stimulator, grid of electrical stimulation chamber, electric stimulation chamber, lateral restraint, vertical base, 43 steps, passage between ladder and resting chamber and electrical stimulation panel. The prototype has 1129 millimeters of the height of the ladder, 1348 millimeters total height, with the base of 300 millimeters wide, with 43 steps of the width of 0.02 millimeters and with a slope of 80 degrees. In addition, it has lateral contentions characterized by 125 mm of height, by 1129 mm of length. The operation of the electric stimulation chamber is performed by a panel of 150 millimeters in height, 288 millimeters in length (with a 30º inclination), 190 millimeters in width, with a round switch of 20 millimeters in diameter and 27.5 millimeters depth switch on and off the electrical stimulator, with two switch buttons 10 mm in diameter and 15 mm in length for intensity and frequency respectively, with a DC\AC inverter circuit, with integrated circuit 555 base, where

it can generate 60 Volts AC. The resting chamber is 219 millimeters high, 200 millimeters long, by 295 millimeters wide (Figure 1a and 1b- Supplementary Material).

Animals care

One hundred and fifty-five healthy female C57BL/6 mice, aged 10 to 12 weeks, with about 20 ± 5 grams of body weight. These animals were purchased from the Department of Biochemistry and Molecular Pharmacology of "*Instituto de Ciências Biológicas (ICB) da Universidade Federal de Minas Gerais (UFMG)*'' and were kept in the "*Centro de Ciências Biológicas e da Saúde da Universidade Estadual de Montes Claros (Unimontes)''*. The mice were submitted to an initial adaptation period (10 days, adequate conditions of ambient temperature (22 ± 2ºC, relative humidity of 60 ± 5%, 12h of light / dark cycles and low sound level) <40 dB, with free access to filtered water and balanced feed (Purina-Labina®) containing 50.3% of carbohydrates, 41.9% of proteins and 7.8% of fat with a total of 2.18 kcal per 1g of feed. The animals were housed in groups of 3 to 5 animals in boxes of autoclavable polypropylene of dimensions of 414 x 344 x 168 mm, with a lid in galvanized steel and containing stainless steel separators (Zootech, model ZT 375. All boxes were lined with shavings, which were changed three times a week, and the animals consumed daily were weighed using an analytical balance (Bonther®, Ribeirão Preto, Brazil) daily. Ingested water was also measured at regular time intervals.

Groups of Animals

The animals were organized into 4 groups as follow: i. control; ii. spontaneous physical activity (SPA); iii. resistance exercise without stimulation by electric shock (RE); iv. resistance exercise with electrical shock stimulation (REE). The control group did not perform any type of intervention, it was in the same housing conditions at the time of the interventions in the

other groups. The SPA group condition was performed in a closed enriched environment (plastic box) with 60 cm in length, 30 cm in width and 45 cm in height (Figure S2). This environment was composed of seesaw, wheel, balls, and tunnels (Coletti et al., 2016; Hutchinson, Avery, & VandeWoude, 2005; Van de Weerd et al., 2002). The RE group condition was performed using a vertical ladder 110 cm high, 18 cm wide, 2 cm between the steps and 80° slope (Figure S1c). REE group condition was performed using the electrostimulation ladder (Figure S1a and S1b). In RE and REE the animals did 6 sets of 8 repetitions with intervals of 90 seconds between the sets and the resistance offered by the exercise was offered by the animal's own body weight, the proposal was adapted according to the literature (Cassilhas et al., 2012; Cassilhas et al., 2013). In REE the animal was stimulated to rise due to the presence of a steel plate at the base of the ladder in which an electric current of 20 volts of intensity and 45 Hz of frequency was applied to the four legs of the animal. In the supplementary material, two videos were provided, showing how spontaneous physical activity occurred, resistance exercise without shock, and resistance exercise with shock. In order to avoid compromising the experimental protocols, two moments of familiarization of the animals were performed in advance of each procedure. The research team performed pilot procedures for technical calibration.

Acute effects of RE, REE, SPA and controls on lactate levels

To evaluate the acute effects promoted by the protocols, 20 animals were randomly assigned to REE (n = 5), RE (n = 5), SPA (n = 5) and control (n = 5). The sessions took place in the afternoon. Before and immediately after the session, peripheral blood samples obtained by puncturing the caudal end of each animal were collected and placed in test strips for lactate quantification (BM-Lactate®, Roche, Rio de Janeiro, Brazil). These test strips containing the blood samples were then introduced into the portable lactate analyzer (BM-Lactate®, Roche,

Rio de Janeiro, Brazil) (Terra, Alves, Gonçalves da Silva, Salerno, & Dutra, 2012). A vessel was used to mobilize the animals (Figure S3).

Evaluation of the chronic effects of RE, REE and SPA on muscle strength

The grip strength tests of the front and back limbs performed in a grip strength meter (Bonther®, Ribeirão Preto, Brazil). The animals were pulled horizontally by the tail until the grip on the bar was broken. This traction occurred at a constant speed and slow enough to allow the animal to try to increase resistance against the dynamometer. The obtained value of muscle strength was then recorded. This procedure was performed 3 times with intervals of 90 seconds between each measurement. The assessment of muscle strength was performed initially for the front limbs and later for the muscular strength obtained with all limbs (De Luca et al., 2008; Takeshita et al., 2017; Velázquez et al., 2014).

It is important to emphasize that the reliability of the muscle strength test was verified, our results showed that the measurement of the muscular strength obtained in the groups of animals evaluated was considered reliable (CI = 0.807) for the evaluation of the muscular tensile strength by the front members ; and (IC = 0.917) to evaluate the tensile strength for all members (Tables S1 and S2, respectively - Supplementary Material) (Portney & Watkins, 2000; Shrout & Fleiss, 1979).

Twenty animals were randomly assigned into 4 groups: REE (n = 5), RE (n = 5), SPA (n = 5) and control (n = 5). The animals were submitted to 10 sessions at 48-hour intervals between sessions. The tensile strength was measured as previously described in groups of animals, 48 hours before the first session and 48 hours after the last session. All sessions were held in the afternoon.

Evaluation of the chronic effects of RE, REE, and SPA on limb volume and body composition

In order to evaluate the short-term chronic effects of RE, REE and SPA on right hind limb volume and body composition of the animals, 12 animals were randomly assigned to 4 groups: REE (n = 3), RE (n = 3), SPA (n = 3) and control (n = 3). The animals were submitted to 10 sessions at 48-hour intervals between sessions. The volume of the right hind limb was measured 48 hours before the first session and 48 hours after the last session. The evaluation of right hind limb volume was performed using ultrasound equipment (G & E Voluson P8 4D) with linear transducer 12L-RS (Frequency: 4.2 – 13.0 MHz). The scanning of the member volume occurred in the network interface (Ethernet) where it enabled documentation in DICOM standard. Prior to this procedure, the animals were anesthetized with ketamine/ xylazine (75 mg/kg and 5 mg/kg body weight, respectively) (Close et al., 1996; Foster, Pavlin, Harasiewicz, Christopher, & Turnbull, 2000; Honors & Kinzig, 2014).

After 24 hours of the last assessment of limb volume, all animals were anesthetized again with ketamine/xylazine (75 mg/kg and 5 mg/kg body weight, respectively) and euthanized by cervical dislocation (Close et al., 1996; Foster et al., 2000; Honors & Kinzig, 2014) to perform the collection of skeletal muscle tissues of the quadriceps, visceral white adipose, and adipose brown scapular. The collected tissues were weighed using an analytical precision digital scale (A. Scientific EEQ9003E) (Hansen, Han, Nolte, Chen, & Holloszy, 1997; Honors & Kinzig, 2014).

In the ultrasound evaluation the reliability was verified, our results showed that this analysis was reliable (CI = 0.856) (Tables S3 - Supplementary Material) (Portney & Watkins, 2000; Shrout & Fleiss, 1979).

Area of skeletal muscle fibers and quantification of nuclei

Skeletal muscle structure features a "cable-within-a-cable" structure, in which individual muscle fibers are aligned in parallel with the length of the tissue and are ensheathed by extracellular matrix proteins. A section of skeletal muscles anterior tibialis and gastrocnemius was cut at 90º to the longitudinal axis. Sections were then examined and photographed using an Olympus BX50 microscope (Olympus Optical). Muscle fiber cross-sectional areas of muscles were determined using Image J software (Scion, Frederick, MD). The greatest distance between the opposite sides of the narrowest aspect of the fiber was chosen (Pallafacchina, Calabria, Serrano, Kalhovde, & Schiaffino, 2002). Measurement of skeletal muscle fibers was performed at least three muscles per group and three distinct randomly chosen fields of each muscle cross section. All data are expressed as the mean ± S.D.

Assessment of the acute and chronic effects of RE, REE and SPA in the exploration of the light-dark box

With the objective of evaluating the acute and chronic effects of RE, REE and SPA in the exploration of the light-dark box. The test consisted of a 5 minute session, the animals were allowed to explore a new environment composed of two different compartments: protected (dark) and unprotected (illuminated) (Campos, Fogaca, Aguiar, & Guimaraes, 2013). They were randomly distributed into 4 groups: REE (n = 3), RE (n = 3), SPA (n = 3) and control (n = 3). The first experiment (acute effect) occurred immediately after the treatment proposal. The second experiment (short-term chronic effect) occurred before and after 5 sessions (at 24 hour intervals between sessions) of the treatment proposal.

Statistical analysis

All data collected were scanned into an electronic database. Subsequently, the data were analyzed statistically in the SPSS software (Statistical Package for the Social Sciences) 20.0. All data are presented as means and standard deviations. The confidence level adopted in all analyzes was set at 95% ($p < 0.05$). The Shapiro-Wilk test were performed to verify normality. After such analysis, paired Student's t-test, independent Student's t-test, Kruskal-Wallis test, the Wilcoxon test, the ANOVA test and the effect size were performed using the Hopkins classification (2009) (Hopkins, Marshall, Batterham, & Hanin, 2009; Nuzzo, 2014).

RESULTS

Acute effects of resistance exercise and spontaneous physical activity on lactate levels.

The acute effects of resistance exercise on plasma lactate levels in animals submitted to REE showed results similar to those obtained with RE animals. There were a significant increase in blood lactate levels before and after physical exercise in the REE ($p = 0.005$), RE ($p = 0.027$) and SPA ($p = 0.011$) groups; in the control group there was no significant difference in mean differences (Table 1).

Short-term chronic effect on muscle strength obtained in the REE group was significantly better than those obtained in the SPA, RE and control groups.

Only the REE mice showed a significant increase in all muscle strength variables investigated in the present study: average absolute muscle strength of the anterior limbs (AAS), average relative muscle strength of the anterior limbs (ARS), average absolute muscle strength of all limbs (AAS4), average relative muscle strength of all members (ARS4), maximum absolute muscle strength of the anterior limbs (MAS), maximum relative muscle strength of the anterior limbs (MRS), maximum absolute muscle strength of the anterior limbs MAS4), and

the maximum relative muscle strength of all limbs (MRS4) ($p < 0.05$). The animals of the RE group did not present significant differences only for the AAS4 and ARS4 muscle strength variables ($p > 0.05$). The animals that performed SPA had no significant difference for any form of muscle strength measurement ($p > 0.05$). In animals in the control group, a significant increase of AAS4, ARS4, MAS4, and MRS4 occurred ($p > 0.05$). In Table 2 the situation described above can be visualized.

Additionally, when delta values were compared between groups, animals from the REE group showed a significant difference with animals from the control group for the AAS variable ($p = 0.045$) and animals from the SPA group for the MAS variable ($p = 0.005$) (Table 3). Due to the small number of animal samples in each group investigated in this study, the size of the effect was processed, all variables presented a large classification (> 1.0), except in the variable AAS4 between SPA and REE that was identified as trivial (-0.02) (Table S4 - Supplementary material).

Short-term chronic effect of REE did not promote an increase in the weight of brown adipose scapular tissue and femoral quadriceps muscle in C57BL / 6 mice compared to values obtained with the animals in SPA, RE, and control.

Animals submitted to REE showed a greater measurement of the total and percentage weights of the adipose brown scapular tissue and the quadriceps femoris muscle (Table 5). However, when compared to data from the other groups of animals investigated, no statistically significant difference was identified.

The short-term chronic effects of REE promote an increase in the volume of the right hind limb in C57BL / 6 mice

Animals submitted to REE presented a greater increase in right back limb volume (Table 4), the results were significant ($p = 0.012$) when compared to moments before and after an intervention. However, when comparing the delta values with the data obtained from the other groups of animals investigated, no statistically significant difference ($p < 0.05$) was identified. However, the size of the effect showed interesting results (Table S5 - Supplementary Material), where it showed between REE vs. Control, REE vs. RE, and REE vs. SPA was respectively classified as perfect (4.29), small (0.27) and almost large (1.02).

Short-term chronic effect of REE promoted a significant increase of nuclei in the muscle fibers in C57BL / 6 mice compared to the values obtained with the animals in SPA, RE, and control.

There was no significant difference (Table 6) between the muscle fiber area (μm^2) of the control, SPA, RE and REE animals. However, the number of nuclei in the skeletal muscle fiber in REE was significantly higher when compared to SPA ($p = 0.000$), RE ($p = 0.003$) and Control ($p = 0.000$).

The acute and chronic short-term effects in the REE animals on the light-dark box did not show significant differences when compared to the animals of the SPA, RE and controls.

The variance of time in the light environment (seconds), time in dark environment (seconds), change of environment (units), total distance traveled in the light environment (centimeters) and average speed (cm / s) were analyzed.

Checking the acute effect (table 7) of the interventions, no statistically significant difference was identified ($p > 0.05$). Regarding the short-term chronic effect, no statistically significant difference ($p > 0.05$) was found in the delta values (table S6) between the groups.

Analyzing the moments (table 8) in each group, there was a significant difference (p = 0.000) in the variable total distance (cm) of the control group, where it presented a decrease between baseline 1 (2510.70 ± 87.22) for baseline 2 (50.16 ± 10.35).

DISCUSSION

The presented prototype study showed important results for future scientific interventions when compare resistance exercise protocols with or without electric stimulation with spontaneous physical activity in an enriched environment. This utility model showed similarities in the production of blood lactate with RE and SPA, decrease in blood glucose, greater increase of muscle strength and in all variables analyzed the greater weight of quadriceps femoris and adipose scapular brown tissue.

Lactate is produced in anaerobiosis, a result of low tissue perfusion (hypoxia), it is an organic compound (byproduct) derived from carbohydrate metabolism, its increase triggers the use of the bioenergetic system known as lactic anaerobic (Brooks, 1991; Spiering et al., 2008). In this study, it was observed that immediately after the exercise session the significant increase of the blood lactate in the REE, RE and SPA groups occurred. The highest increase occurred in RE and values close to REE, showing the characterization of the training of these studies has a predominantly lactic anaerobic specification, a fact that helps to describe resistance exercise with the goal of muscular hypertrophy, muscular power and/or muscular strength (Medicine, 2002; Ratamess et al., 2009). Corroborating with the findings, a study examining several resistance exercise methods for humans aimed at increasing muscle strength and hypertrophy, found significant increases in blood lactate (Gentil et al., 2006). Another study reinforces the specificity of our research, using 24 male Wistar rats, the animals were distributed in 4 groups: untrained, resistance training, hypertrophy training and strength training. The proposal lasted 12 weeks, moments of acute analysis of blood lactate levels, our variable of interest, the results

of this immediate effect showed that blood lactate increased in the experimental groups, this increase is lower after the 12-week chronic adaptation (Scheffer et al., 2012).

RES animals were stimulated with shock in the exit chamber of the ladder, causing a jump (videos available in supplementary materials) between the sixth and the tenth step, biomechanically the animal used with greater expressiveness the hind limbs to execute the exit of the camera due to this electric impulse. Thus, it was observed that the development of muscular strength in the RES group was higher among the groups, a fact that can be explained by the adaptations resulting from the better overload imposed on the organism of these animals, possibly obtaining an improvement in neural factors, such as the progression of intramuscular and intermuscular coordination, contraction efficiency and relaxation (co-activation) and improvement of the bilateral deficit (Haff & Triplett, 2015; Medicine, 2002; Ratamess et al., 2009). In the present study we evaluated the functional and muscular effects of two methods of strength training, burrowing and tower unloading, in male C57B16 mice. To compare these two innovative methods with existing exercise methods, resistance and run (no resistance) functioning were included. Engine coordination, grip strength, and muscle fatigue were measured, the experiment lasted fourteen weeks. It was noticed the result of the muscular force of traction in the method burrowing, functionally improved in comparison with the controls (Roemers et al., 2017). Although the method is different from the ones proposed in our study, it is important to show that the existence of adaptations of muscular strength in the intentionality proposals that exist in the literature about the model of experimental resistance exercise has the occurrence of increased muscle strength.

After the neural adaptation, the process of hypertrophy becomes more evident, in the results of the body composition the quadriceps femoral muscle and the brown adipose tissue present higher absolute and percentage weights in the animals of the RES group, except in the comparison with the SPA that had the same percentage of brown adipose tissue. The femoral

quadriceps has the agonist function in the movement (leg extension) related to REE and RE (Dyce, Sack, & Wensing, 2009; Dyce, Wensing, & Sack, 2004; König & Liebich, 2016; Netter, 2008). However, two findings draw attention to the REE. The first finding is the largest significant increase in right back limb volume found, consistently the second finding is the highest number of nuclei in the muscle fiber in these REE animals. These results show that the potential of the hypertrophic adaptations in the REE methodological proposal is positive indicators of the model presented here. The increase in the number of nuclei is related to the satellite cells, in the face of the appropriate stimulus, they proliferate and fuse with the muscle fibers or with themselves, this is one of the situations that provide muscular hypertrophy (Kadi & Thornell, 2000). It is important to note that the duration of the experiments were three weeks with a total of 10 sessions, the significant hypertrophic adaptations in humans, usually occur between 8 to 12 weeks of resistance training (Medicine, 2002; Ratamess et al., 2009; Spiering et al., 2008; Zatsiorsky & Kraemer, 2006). Such comparisons with the scientific literature reinforce the results found here but show a potential for the advancement of new studies with longer duration.

Regarding the brown adipose tissue that presented higher values in RES but without significant difference, this tissue is important for thermogenic response and energetic balance in the organic system, its action contributes to avoiding obesity (Klingenspor et al., 2017). The results may be related to the training format, but it is not possible to state since the findings are a cross-sectional characterization performed at the end of the study. In this aspect, we have a limitation, but a research potential is shown since the presented values go according to the effects of the exercise in these variables of the body composition of the animals (De Matteis et al., 2013; Medicine, 2002; Ratamess et al., 2009; Spiering et al., 2008; Stanford, Middelbeek, & Goodyear, 2015).

Activity and physical exercise, in general, are important non-pharmacological strategies for the prevention of behavioral disorders (Fulk et al., 2004). However, considering the new methodological strategy proposed in this study, we are concerned about the possibility of behavioral changes, a fact that may directly implicate the aggravation of several other health conditions (Campos et al., 2013). The results of the acute effects show that there is no difference in behavior in the different interventions proposed. It is reported that the acute effects of physical exercise can cause for several hours the decrease in anxiety levels (Raglin & Wilson, 1996). Anxiolytic drugs, for example, showed the same test (light-dark test), more time spent in the lighted environment and a greater number of transitions between the two areas (Crawley, Marangos, Paul, Skolnick, & Goodwin, 1981), and a fact related to our findings (table 7). Higher values of transitions and in the light environment occurred in animals submitted to REE, RE, and SPA.

Regarding the descriptive data related to the chronic short-term effect (Table 7 and Table S6), there were no significant changes in the analyzed variables of the animals submitted to REE, RE and SPA, showing that the implication of the activity and the physical exercise, in particular the resistance training did not have negative impact, revealing the possible maintenance of the behavioral variables (Morgan, 1985), even with interventions that involve physiological changes affecting the organism to the stable state.

In the control group, the total distance (cm) covered in the light-dark scanning test decreased significantly ($p = 0.000$). The modifications of routines and environment, allow the reduction of behavioral changes, improving the health conditions and reproductive performance (Newberry, 1995), a fact not experienced in this group, allowing a differentiated behavior when compared to the groups submitted to the innervations.

FINAL CONSIDERATIONS AND CONCLUSIONS

This research presented important results on the use of the vertical ladder device for resistance exercise with or without electrical shock stimulation, since the findings show that GER presented similarities in the acute and chronic adaptations expected for the resistance training. The data presented here are the first notes of the use of this utility model. The methodology presented here offers promising perspectives in research related to skeletal muscle structure, leading to propositions related to physical fitness related to health and/or performance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could influence the impartiality of the research reported.

ACKNOWLEDGEMENTS

We would like to thank the Foundation for Research Support of the Minas Gerais State (FAPEMIG; grant numbers: PPM-00029-17 and DEG-00010-16); National Council for Scientific and Technological Development (CNPq, grant number: 150038/2017-8 and 430759/2016-9); and the Coordination of Improvement of Higher Education Personnel (CAPES) for the support to carry out this study. AMB De-Paula is research productivity fellow of the CNPq.

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Tables

Table 1. Mean and standard deviation values of blood lactate levels before and after the resistance exercise with electrostimulation (RES), resistance exercise without electrical stimulator (RE), and spontaneous physical activity (SPA).

Blood Level	Control (n = 5)				REE (n = 5)				RE (n = 5)				SPA (n = 5)			
	Baseline 1	Baseline 2	Delta value(Δ)	p value	Before session	After session	Delta value(Δ)	p value	Before session	After session	Delta value(Δ)	p value	Before session	After session	Delta value(Δ)	p value
Lactate (mmol/L) (mean ± S.D.)	6.90 ± 0.47	6.90 ± 0.48	0.10 ± 0.08	0.099	6.14 ± 0.16	7.26 ± 0.37	1.12 ± 0.438	0.005*	7.28 ± 0.54	9.16 ± 1.22	1.88 ± 1.26	0.027*	7.16 ± 0.44	8.18 ± 0.67	1.02 ± 0.51	0.011*

*The diferences were statistically significant. Means of groups were compared using paired Student's T test. S.D. = standard deviation. REE = the resistance exercise with electrostimulation. RE = resistance exercise without electrical stimulator. SPA = spontaneous physical activity.

Table 2. Mean and standard deviation values of the various muscle strength variables obtained before and after the use of resistance exercise with electrostimulation (RES), resistance exercise without electrical stimulator (RE), and spontaneous physical activity (SPA), and control.

Variables	Groups											
	Control (n = 5)			REE (n = 5)			RE (n = 5)			SPA (n = 5)		
	Baseline 1	Basiline 2	p value	Before sessions	After sessions	p value	Before sessions	After sessions	p value	Before sessions	After sessions	p value
AAS (g)	82.67 ± 13.07	76 ± 10.34	0.637	64.33 ± 9.28	93.67 ± 1.78	0.007*	76.33 ± 10.29	88 ± 6.02	0.008*	79.67 ± 13.01	82.67 ± 15.92	0.738
ARS (g/g)	3.87 ± 0.50	3.67 ± 0.46	0.589	2.96 ± 0.51	4.46 ± 0.64	0.009*	3.5 ± 0.54	4.29 ± 0.29	0.007*	4.06 ± 0.55	3.9 ± 0.94	0.598
AAS4 (g)	104 ± 7.35	121.33 ± 8.38	0.009*	127.33 ± 0.74	154 ± 9.55	0.011*	131 ± 2.52	144.67 ±9.86	0.154	135.33 ± 9.02	162.33 ± 23.03	0.331
ARS4 (g/g)	4.81 ± 0.37	5.81 ± 0.41	0.006*	5.49 ± 0.61	7.32 ± 0.57	0.017*	5.58 ± 0.21	6.7 ± 0.34	0.057	7.01 ± 0.92	7.66 ± 1.24	0.180
MAS (g)	89 ± 15.65	89 ± 12.38	0.685	71 ± 13.27	111 ± 12.56	0.026*	80 ± 7.52	105 ± 6.54	0.006*	100 ± 9.55	84 ± 16.81	0.253
MRS (g/g)	4.54 ± 0.64	4.2 ± 0.63	0.694	3.25 ± 0.70	5.34 ± 0.50	0.028*	3.67 ± 0.43	4.91 ± 0.34	0.006*	5.18 ± 0.41	4.49 ± 1.05	0.408
MAS4(g)	117 ± 14.23	135 ± 10.57	0.013*	124 ± 11.13	178 ± 11.05	0.001*	134 ± 1.95	152 ± 11.26	0.011*	154 ± 22.81	185 ± 42.28	0.205
MRS4 (g/g)	5.68 ± 0.78	6.46 ± 0.53	0.013*	5.57 ± 0.71	8.38 ± 0.60	0.002*	6.01 ± 0.2	7.39 ± 0.38	0.004*	7.4 ± 1.15	8.73 ± 2.34	0.164

* The diferences were statistically significant. Means of groups were compared using paired Student's T test. S.D. = standard deviation. REE = resistance exercise with electrostimulation. RE = resistance exercise. SPA = spontaneous physical activity. AAS = average absolute muscle for anterior limbs. ARS = average relative muscle strength for anterior limbs. AAS4 = average absolute muscle strength for all limbs. ARS4 = average relative muscle strength of all limbs. MAS = maximum absolute muscle strength for anterior limbs. MRS = maximum relative muscle strength for anterior limbs. MAS4 = maximum absolute muscle strength for all limbs. MRS4 = maximum relative muscle strength of all limbs.

Table 3. Mean values and delta standard deviation (Δ) for muscle strength assessment before and after short-term chronic response of control, resistance exercise with eletrostimulation (RES), resistance exercise (RE), e spontaneous physical activity (SPA) groups.

Variables	Control (n = 5)	REE (n = 5)	RE (n = 5)	SPA (n = 5)	REE vs. Control p value	REE x RE p value	REE vs. SPA p value
AAS (g)	-3.20 ± 14.06	25.46 ± 11.29	12.06 ± 5.59	3.66 ± 22.89	0.045*	1.000	0.202
ARS (g/g)	-0.14 ± 0.57	1.2 ± 0.56	0.69 ± 0.3	0.3 ± 1.19	0.060	1.000	0.424
AAS4 (g)	24.06 ± 11.21	35.33 ± 17.42	8.66 ± 11.04	8.66 ± 17.51	1.000	0.065	0.066
ARS4 (g/g)	1.11 ± 0.46	1.67 ± 0.94	0.62 ± 0.52	0.6 ± 0.83	1.000	0.202	0.187
MAS (g)	3 ± 15.37	31.4 ± 18.60	18.69 ± 7.63	-10.55 ± 18.10	0.079	1.000	0.005*
MRS (g/g)	0.14 ± 0.75	1.46 ± 0.97	1.01 ± 0.42	-0.4 ± 0.97	0.120	1.000	0.013
MAS4(g)	23.4 ± 12.34	53.6 ± 14.67	21.8 ± 10.75	26.4 ± 38.98	0.292	0.235	0.436
MRS4 (g/g)	1.07 ± 0.56	2.52 ± 0.80	1.24 ± 0.46	1.53 ± 2.01	0.375	0.577	1.000

* The diferences were statistically significant. Mean and standard deviation (S.D.) bof the group delta values were compared using analysis of variance variance (ANOVA) Bonferroni setting. = resistance exercise with electrostimulation. RE = resistance exercise without electrical stimulator. SPA = spontaneous physical activity. AAS = average absolute muscle for anterior limbs.ARS = average relative muscle strength for anterior limbs. AAS4 = average absolute muscle strength for all limbs. ARS4 = average relative muscle strength of all limbs. MAS = maximum absolute muscle strength for anterior limbs. MRS = maximum relative muscle strength for anterior limbs. MAS4 = maximum absolute muscle strength for all limbs. MRS4 = maximum relative muscle strength of all limbs.

Table 4. Analysis of back limb volume (cm^3) right before and after the training.

	Volume of right hind limb (cm^3)			
	Before Training (Mean ± S.D.)	After Training (Mean ± S.D.)	p value	Delta value
Control (n = 3)	0,58 ± 0,07	0,84 ± 0,06	0,085	0,22 ± 0,14
REE (n = 3)	0,41 ± 0,07	0,97 ± 0,07	0,012	0,60 ± 0,11
RE (n = 3)	0,52 ± 0,14	1,05 ± 0,10	0,061	0,57 ± 0,24
SPA (n = 3)	0,48 ± 0,12	0,94 ± 0,04	0,035	0,52 ± 0,15

Volume of right hind limb from the groups were compared using the Wilcoxon test. The delta values were analyzed analysis of variance variance (ANOVA) Bonferroni setting, these data did not have statistically significant differences. S.D. = standard deviation. REE = resistance exercise with electrostimulation. RE = resistance exercise without electrical stimulator. SPA = spontaneous physical activity.

Table 5. Analysis of components from body composition of C57BL/6 mice from resistance exercise with electrostimulation (RES), resistance exercise without electrical stimulator (RE), and spontaneous physical activity (SPA), and control groups after ten sessions of intervention.

Variables	Groups			
	Control (n = 3) Mean ± S.D.	REE (n = 3) Mean ± S.D.	RE (n = 3) Mean ± S.D.	SPA (n = 3) Mean ± S.D.
Scapular brown adipose tissue (g)	0,078 ± 0,056	0,341 ± 0,447	0.094 ± 0.048	0,104 ± 0,028
Scapular brown adipose tissue (%)	0,376 ± 0,265	1,562 ± 2,067	0.382 ± 0.176	0,473 ± 0,107
Visceral white adipose tissue (g)	0,213 ± 0,146	0,287 ± 0,115	0,343 ± 0,116	0,315 ± 0,145
Visceral white adipose tissue (%)	1,004 ± 0,610	1,316 ± 0,566	1,447 ± 0,564	1,465 ± 0,725
Femur quadriceps muscle (g)	0,167 ± 0,013	0,372 ± 0,448	0.143 ± 0.033	0,162 ± 0,043
Femur quadriceps muscle (%)	0,811 ± 0,111	1,705 ± 2,075	0.595 ± 0.131	1,705 ± 2,075
Gastrocnemius muscles and soleus (g)	0,246 ± 0,088	0,223 ± 0,069	0,195 ± 0,059	0,246 ± 0,059
Gastrocnemius muscles and soleus (%)	1,207 ± 0,520	1,024 ± 0,361	0,823 ± 0,298	1,130 ± 0,312

All diferences were not statistically significant ($p < 0,05$). Groups were compared using using Kruskal-Wallis test. S.D. = standard deviation. REE = resistance exercise with electrostimulation. RE = resistance exercise without electrical stimulator. SPA = spontaneous physical activity.

Table 6. Analysis of the cross-sectional area dimensions of the muscle fibers of C57BL / 6 mice of resistive exercise with electrostimulation (REE), resistance exercise without electrical stimulator (RE) and spontaneous physical activity (SPA) and control groups after ten intervention sessions.

Variables	Femur quadriceps muscle				p value		
	Control (n = 3) Mean ± S.D.	REE (n = 3) Mean ± S.D.	RE (n = 3) Mean ± S.D.	SPA (n = 3) Mean ± S.D.	REE vs.Control	REE x RE	REE vs. SPA
Average number of nucleus	15,44 ± 3,74	53,66 ± 10,57	35,55 ± 14,75	27,22 ± 7,82	0.000	0.003	0.000
Average fiber area (μm²)	3443,46 ± 725,21	3464,33 ± 781,90	3456,83 ± 670,71	3242,01 ± 666,91	1.000	1.000	0.086
	Absolute value				Non-performing inferential test		
Maximum fiber area (μm²)	4826,00	4795,00	4795,00	4699,00			
Minimum fiber area (μm²)	2413,00	2398,00	2398,00	2390,00			

Variables	Gastrocnemius muscles and soleus				p value		
	Control (n = 3) Mean ± S.D.	REE (n = 3) Mean ± S.D.	RE (n = 3) Mean ± S.D.	SPA (n = 3) Mean ± S.D.	REE vs.Control	REE x RE	REE vs. SPA
Average number of nucleus	33,66 ± 12,35	35,22 ± 10,42	37,88 ± 16,30	39,77 ± 22,33	1.000	1.000	1.000
Average fiber area (μm²)	3401,96 ± 705,14	3525,32 ± 768,88	3356,46 ± 917,20	3401,96 ± 917,20	1.000	1.000	1.000
	Absolute value				Non-performing inferential test		
Maximum fiber area (μm²)	4795,00	4603,00	4699,00	4699,00			
Minimum fiber area (μm²)	2398,00	2398,00	2398,00	2398,00			

Mean and standard deviation (S.D.) bof the group were compared using analysis of variance variance (ANOVA) Bonferroni setting. REE = resistance exercise with electrostimulation. RE = resistance exercise without electrical stimulator. SPA = spontaneous physical activity.

Table 7. Analysis of the immediate behavior of C57BL/6 mice of resistance exercise with electrical stimulation (REE), resistance exercise without electrical stimulator (RE) and spontaneous physical activity (SPA) and control group.

Variables	Groups			
	Control (n = 3) Mean ± S.D.	REE (n = 3) Mean ± S.D.	RE (n = 3) Mean ± S.D.	SPA (n = 3) Mean ± S.D.
Time in the light environment (s)	95.66 ± 27.13	109.66 ± 33.08	135.66 ± 33.00	128.66 ± 22.03
Time in dark environment (s)	204.33 ± 27.13	190.33 ± 33.08	164.33 ± 33.00	171.33 ± 22.03
Change of environment (units)	4.66 ± 1.52	7.33 ± 3.05	6.00 ± 0.00	10.00 ± 2.00
Total distance (cm)	16238.33 ± 3003.57	19502.66 ± 5903.63	18292.00 ± 4035.75	22866.66 ± 4293.21
Average speed (cm/s)	53.66 ± 10,01	65.00 ± 19.92	60.66 ± 13.31	72.66 ± 15.94

All diferences were not statistically significant ($p < 0,05$). Groups were compared using using Kruskal-Wallis test. S.D. = standard deviation. REE = resistance exercise with electrostimulation. RE = resistance exercise without electrical stimulator. SPA = spontaneous physical activity.

Table 8. Analysis of behavior of C57BL/6 mice of resistance exercise with electrical stimulation (REE), resistance exercise without electrical stimulator (RE) and spontaneous physical activity (SPA) and control group after 5 sessions of each intervention.

Variables	Groups											
	Control (n = 3) Mean ± S.D.			REE (n = 3) Mean ± S.D.			RE (n = 3) Mean ± S.D.			SPA (n = 3) Mean ± S.D.		
	Baseline 1	Baseline 2	p	Before sessions	After sessions	p	Before sessions	After sessions	p	Before sessions	After sessions	p
Time in the light environment (s)	112.00 ± 50.23	120.66 ± 151.41	0.947	167.00 ± 45.90	114.33 ± 6.42	0.163	135.00 ± 48.56	188.33 ± 90.51	0.535	153.00 ± 10.14	94.00 ± 45.00	0.105
Time in dark environment (s)	188.00 ± 50.53	179.33 ± 151.41	0.947	133.00 ± 45.90	185.66 ± 6.42	0.163	165.00 ± 48.56	111.66 ± 90.51	0.535	147.00 ± 10.14	206.00 ± 45.13	0.105
Change of environment (units)	9.66 ± 6.02	1.66 ± 1.15	0.186	4.66 ± 1.15	8.000 ± 2.00	0.130	6.33 ± 3.21	7.00 ± 5.56	0.874	5.66 ± 2.08	8.00 ± 3.00	0.192
Total distance (cm)	2510.70 ± 87.22	50.16 ± 10.35	0.000*	2954.86 ± 544.87	2116.96 ± 114.46	0.158	2472.16 ± 411.44	2273.03 ± 1838.14	0.886	2582.16 ± 177.41	1883.76 ± 948.98	0.323
Average speed (cm/s)	8.36 ± 0.30	3.20 ± 3.50	0.112	9.86 ± 1.85	7.06 ± 0.40	0.164	8.26 ± 1.37	7,56 ± 6.12	0.880	8.63 ± 0.58	5.96 ± 2.82	0.227

The Wilcoxon test was used for inferential analysis. S.D. = standard deviation. REE = resistance exercise with electrostimulation. RE = resistance exercise without electrical stimulator. SPA = spontaneous physical activity.

SUPPLEMENTARY MATERIAL

Tables

Table S1. Results of the intrarater reliability test for muscular strength measurement of the anterior limbs in C57BL/6 mice using grip strength meter device.

	n	Test-retest amount	Intrarater correlation	p
Single Measures	51	3	0.807	0.000

Table S2. Results of the intrarater reliability test for muscular strength measurement of the all limbs in C57BL/6 mice using grip strength meter device.

	n	Test-retest amount	Intrarater correlation	p
Single Measures	41	2	0.917	0.000

Table S3. Results of the intra-examiner reliability test for measurement of hindleg volume in C57BL/6 mice using an ultrasound.

	n	Test-retest amount	Intrarater correlation	p
Single Measures	32	2	0.856	0.000

Table S4. Effect size of the mean values and standard deviation of the various muscle strength obtained before and after the use of resistance exercise with electrostimulation (RES), resistance exercise without electrical stimulator (RE), and spontaneous physical activity (SPA), and control.

Variables	Groups					
	REE *vs.* Control		REE *vs.* RE		REE *vs.* SPA	
	Effect size	Classification	Effect size	Classification	Effect size	Classification
AAS (g)	3.18	Very large	1.8	Large	2.22	Very large
ARS (g/g)	3.37	Very large	1.35	Large	3.13	Very large
AAS4 (g)	1.01	Moderate	1.67	Large	-0.02	Trivial
ARS4 (g/g)	12.34	Perfect	2.59	Very large	1.51	Large
MAS (g)	2.76	Very large	1.39	Large	1.3	Large
MRS (g/g)	3.59	Very large	1.49	Large	4.85	Perfect
MAS4(g)	2.82	Very large	4.63	Perfect	1.28	Large
MRS4 (g/g)	2.74	Very large	2.74	Very large	1.55	Large

Classification of effect size was performed using Hopkins (2009) criteria. REE = resistance exercise with electrostimulation. RE = resistance exercise. SPA = spontaneous physical activity. ARS = average relative muscle strength for anterior limbs. AAS4 = average absolute muscle strength for all limbs. ARS4 = average relative muscle strength of all limbs. MAS = maximum absolute muscle strength for anterior limbs. MRS = maximum relative muscle strength for anterior limbs. MAS4 = maximum absolute muscle strength for all limbs. MRS4 = maximum relative muscle strength of all limbs.

Table S5. Size of effect of the hind limb volume (ml) in the animals from obtained before and after the use of resistance exercise with electrostimulation (RES), resistance exercise without electrical stimulator (RE), and spontaneous physical activity (SPA), and control.

Muscle thickness of the hind limb volume (ml)					
Groups					
REE *vs.* Control		REE *vs.* RE		REE *vs.* SPA	
Effect size	Classification	Effect size	Classification	Effect size	Classification
4,29	Almost perfect	0,27	little	1,02	between moderate to large

Classification of effect size was performed using Hopkins (2009) criteria. REE = resistance exercise with electrostimulation. RE = resistance exercise. SPA = spontaneous physical activity.

Table S6. Mean values and delta standard deviation (Δ) for behavioral assessment before and after 5 sessions of intervention, resisted exercise with electrostimulation (REE), resistance exercise (RE) and spontaneous physical activity groups (SPA).

Variables	Groups			
	Control (n = 3) Mean ± S.D.	REE (n = 3) Mean ± S.D.	RE (n = 3) Mean ± S.D.	SPA (n = 3) Mean ± S.D.
Time in the light environment (s)	8.66 ± 201.59	-52.66 ± 42.09	23.33 ± 75.08	-59.00 ± 36.09
Time in dark environment (s)	- 8.66 ± 201.59	52.66 ± 42.09	- 23.33 ± 75.08	59.00 ± 36.09
Change of environment (units)	-8.00 ± 7.00	3.33 ± 2.30	0.66 ± 6.42	2.33 ± 2.08
Total distance (cm)	-2460.53 ± 80.68	-837.90 ± 657.62	-199.13 ± 2120.20	-698.40 ± 928.63
Average speed (cm/s)	-5.16 ± 3.26	-2.66 ± 2.68	-0.70 ± 7.06	-2.80 ± 2.25

Not all differences were statistically significant. Groups were compared using Kruskal-Wallis test. S.D. = standard deviation. REE = resistance exercise with electrostimulation. RE = resistance exercise without electrical stimulator. SPA = spontaneous physical activity.

Figures

Figure S1. The equipment is similar in design to a typical ladder to perform resistant rodent physical exercises (Figure 1c - Barrier OF activities for rodents sold by BONTHER company of the city of Riberão Preto, Brazil). The resistive exercise ladder with electric stimulator (Figure 1a and 1b) for rodents is an instrument that is presented in the form of vertical ladder with containment grid, feeding chamber, resting chamber, support column, horizontal ladder base, stimulator electric, electric stimulation chamber grid, electric stimulation chamber, lateral containment, vertical base, with 43 steps, passage between the ladder and the resting chamber and electrical stimulation panel. The prototype has 1129 millimeters of height of the ladder, 1348 millimeters of total height, with the base of 300 millimeters wide, with 43 steps of the width of 0.02 millimeters and with an inclination of 80 degrees. In addition, it has lateral contours characterized by 125 mm of height, by 1129 mm of length. The operation of the electric stimulation chamber is performed by a panel of 150 millimeters in height, 288 millimeters in length (with a 30º inclination), 190 millimeters in width, with a round switch of 20 millimeters in diameter and 27.5 millimeters in depth. with two buttons 10 mm in diameter and 15 mm in length for intensity and frequency respectively, with a DC \ AC inverter circuit, with a 555 integrated circuit base, where it can generate AC voltages of 60 Volts. The resting chamber is 219 millimeters high, 200 millimeters long by 295 millimeters wide.

Figure S2. Enriched environment for the practice of spontaneous physical activity of rodents using a closed plastic box with 60 cm in length, 30 cm in width and 45 cm in height. This environment was composed of seesaw, wheel, balls and tunnels.

Figure S3. Containment of the animal for biochemical evaluation

Figure S4. Test of muscular tensile strength of the limbs and four limbs

Figure S5. Cross section of femoral quadriceps muscle and gastrocnemius and soleus muscles of mice from control (n = 3), REE (n = 3), RE (n = 3), and SPA (n = 3) groups, after the interventions.

Figure S6. Presentation of ultrasound images of the hind limb volume (ml) of mice from control (n = 3), REE (n = 3), RE (n = 3), and SPA (n = 3) groups, before and after the interventions.

Figure S7. Behavior of the C57BL/6 mouse pathway (acute effect) after resistive exercise with electrical stimulation (REE), resistance exercise without electrical stimulator (ER) and spontaneous physical activity (SPA) and control group.

Figure S8. Behavior of the C57BL/6 mouse trajectory after 5 sessions of resistive exercise with electrical stimulation (REE), resistance exercise without electrical stimulator (ER) and spontaneous physical activity (SPA) and control group.

Figure S1

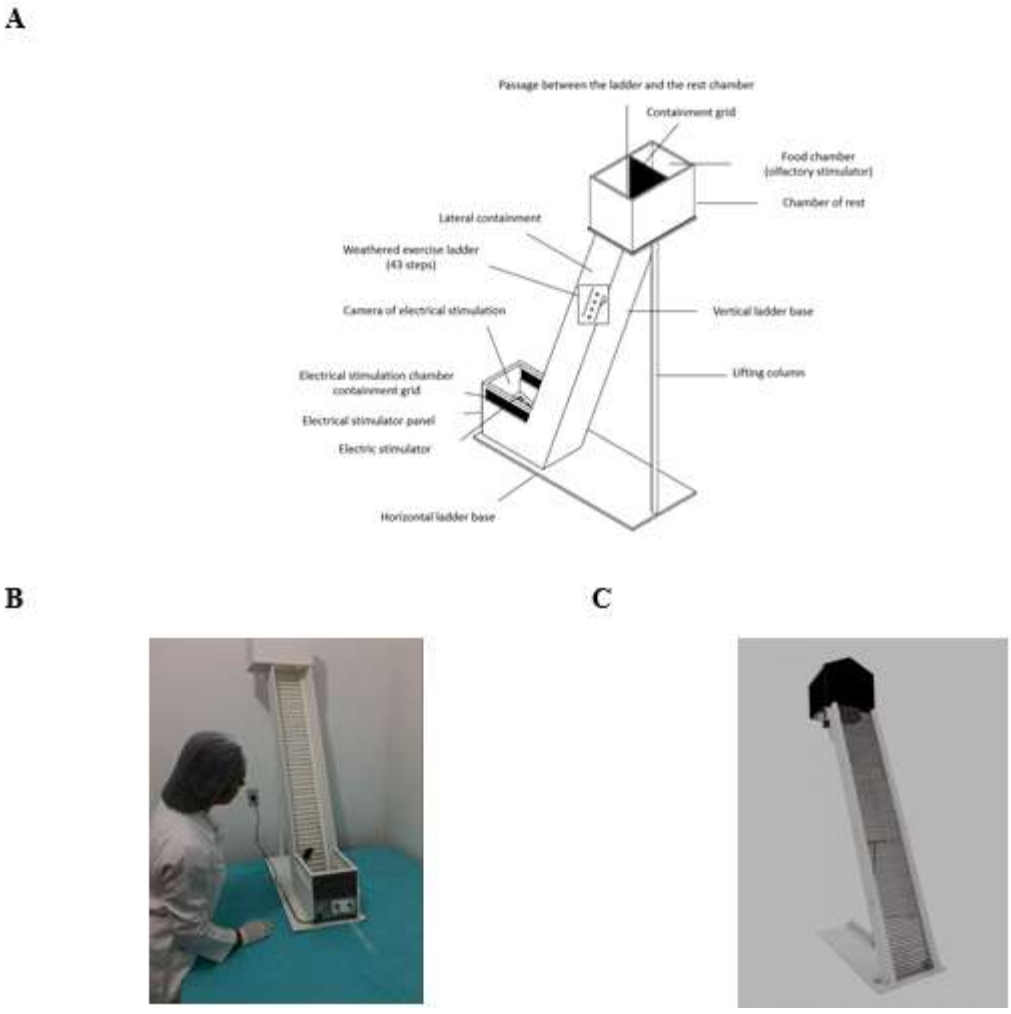


Figure S2

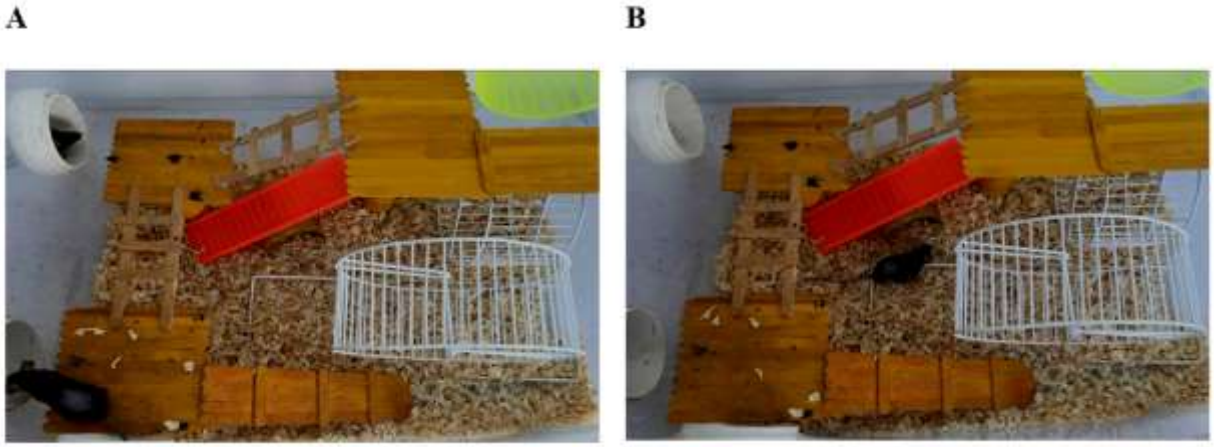


Figure S3

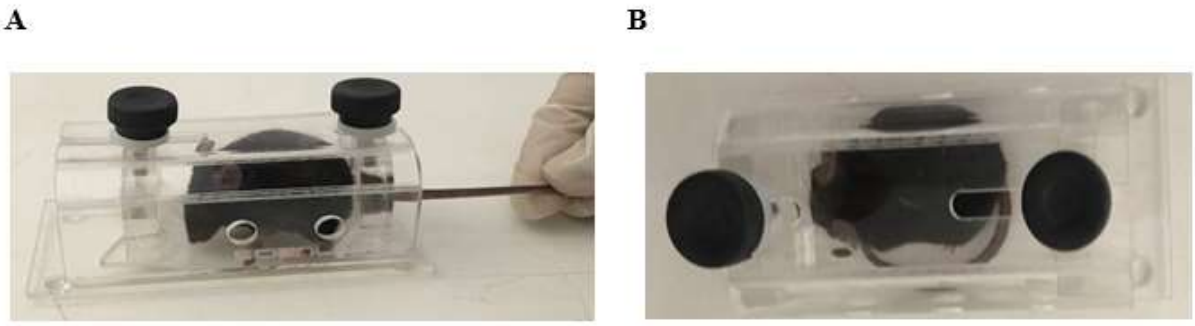
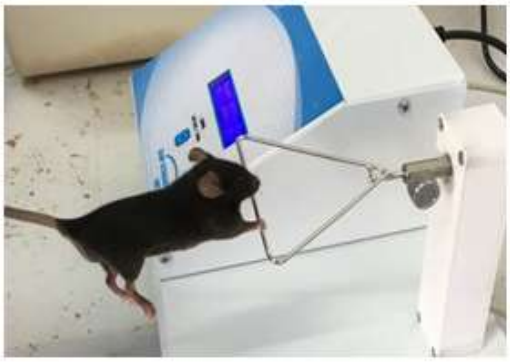


Figure S4

A



B

Figure S5

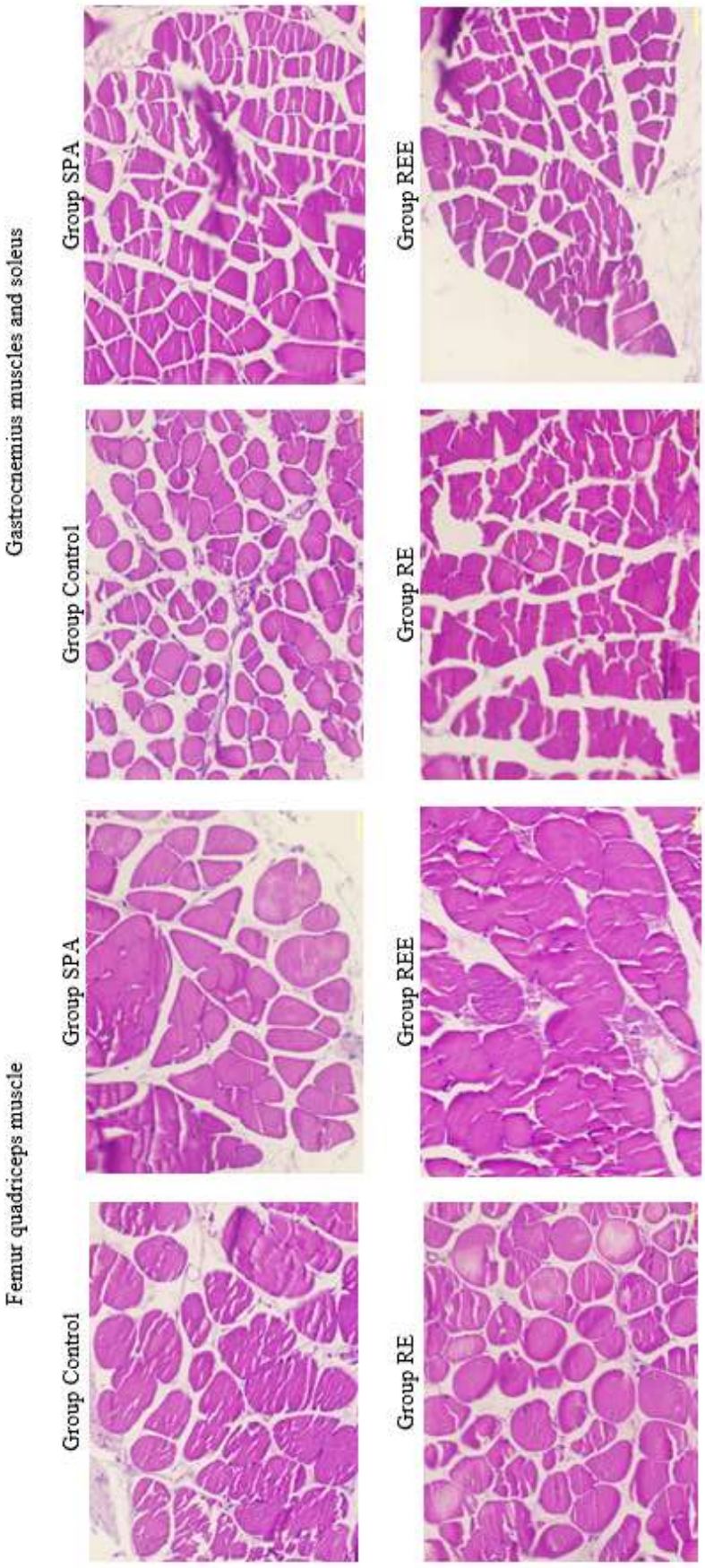

Gastrocnemius muscles and soleus
Group Control
Group SPA
Group RE
Group REE
Femur quadriceps muscle
Group Control
Group SPA
Group RE
Group REE

Figure S6

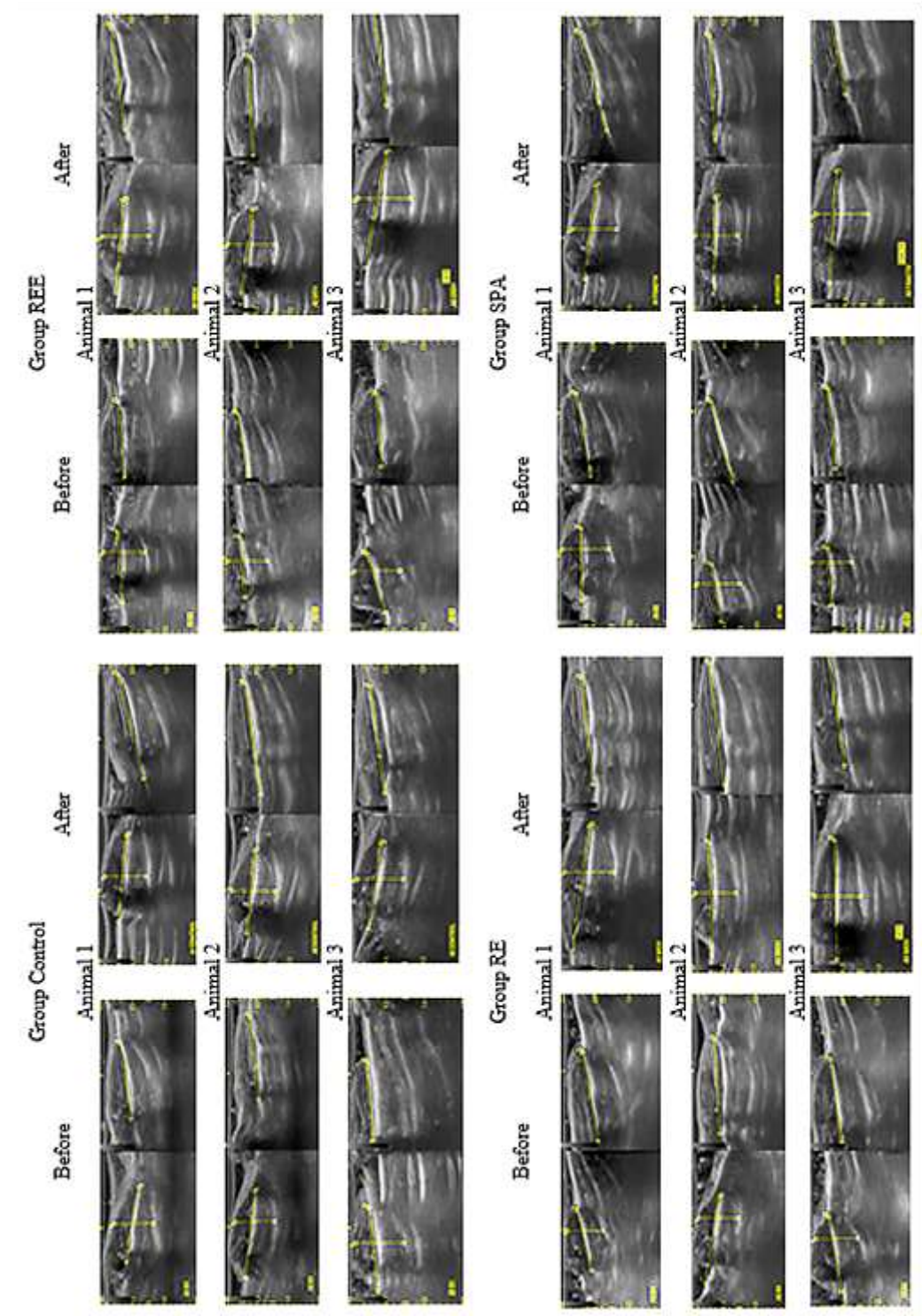

Group Control
Before
After
Animal 1
Animal 2
Animal 3
Group REE
Before
After
Animal 1
Animal 2
Animal 3
Group RE
Before
After
Animal 1
Animal 2
Animal 3
Group SPA
Before
After
Animal 1
Animal 2
Animal 3

Figure S7

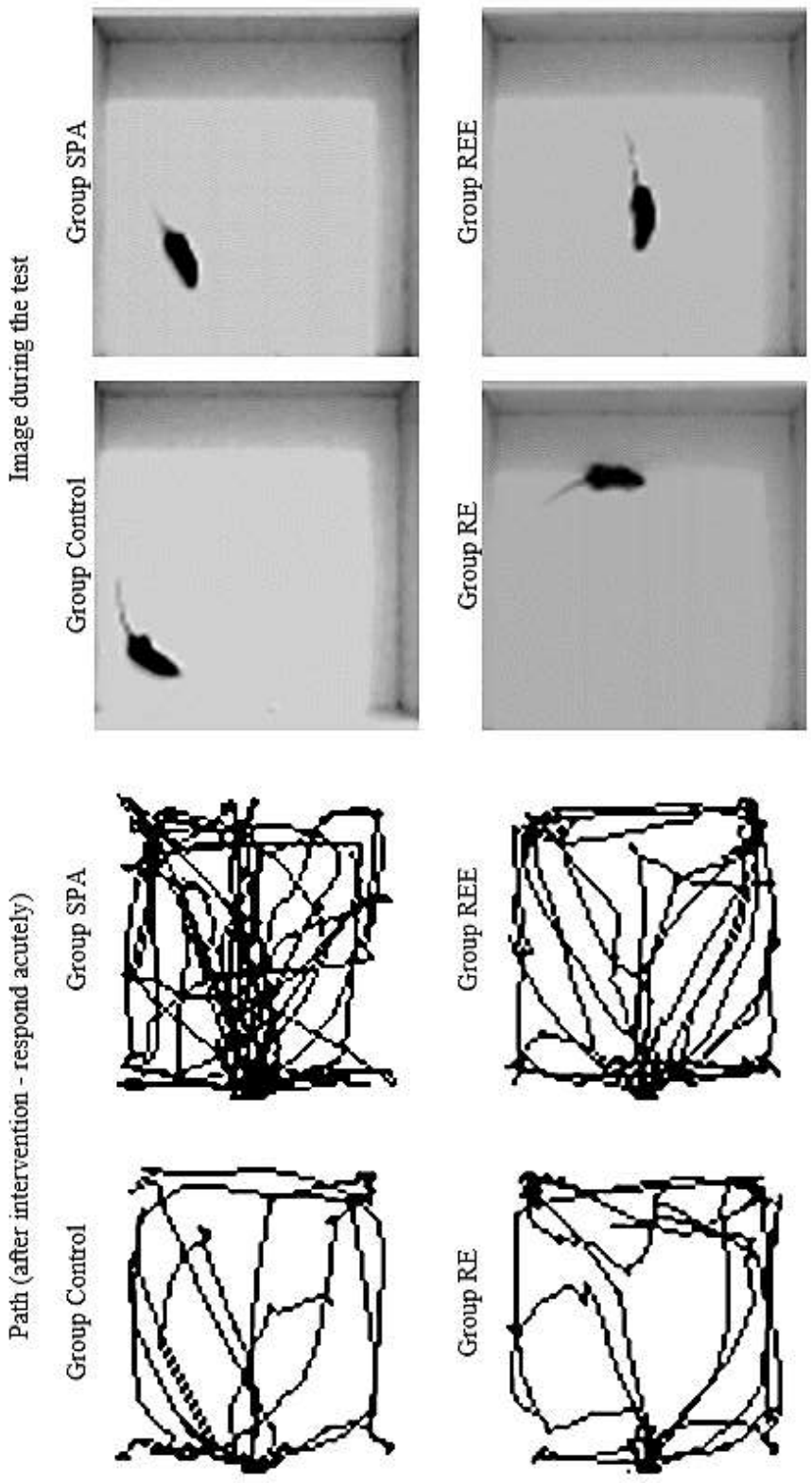
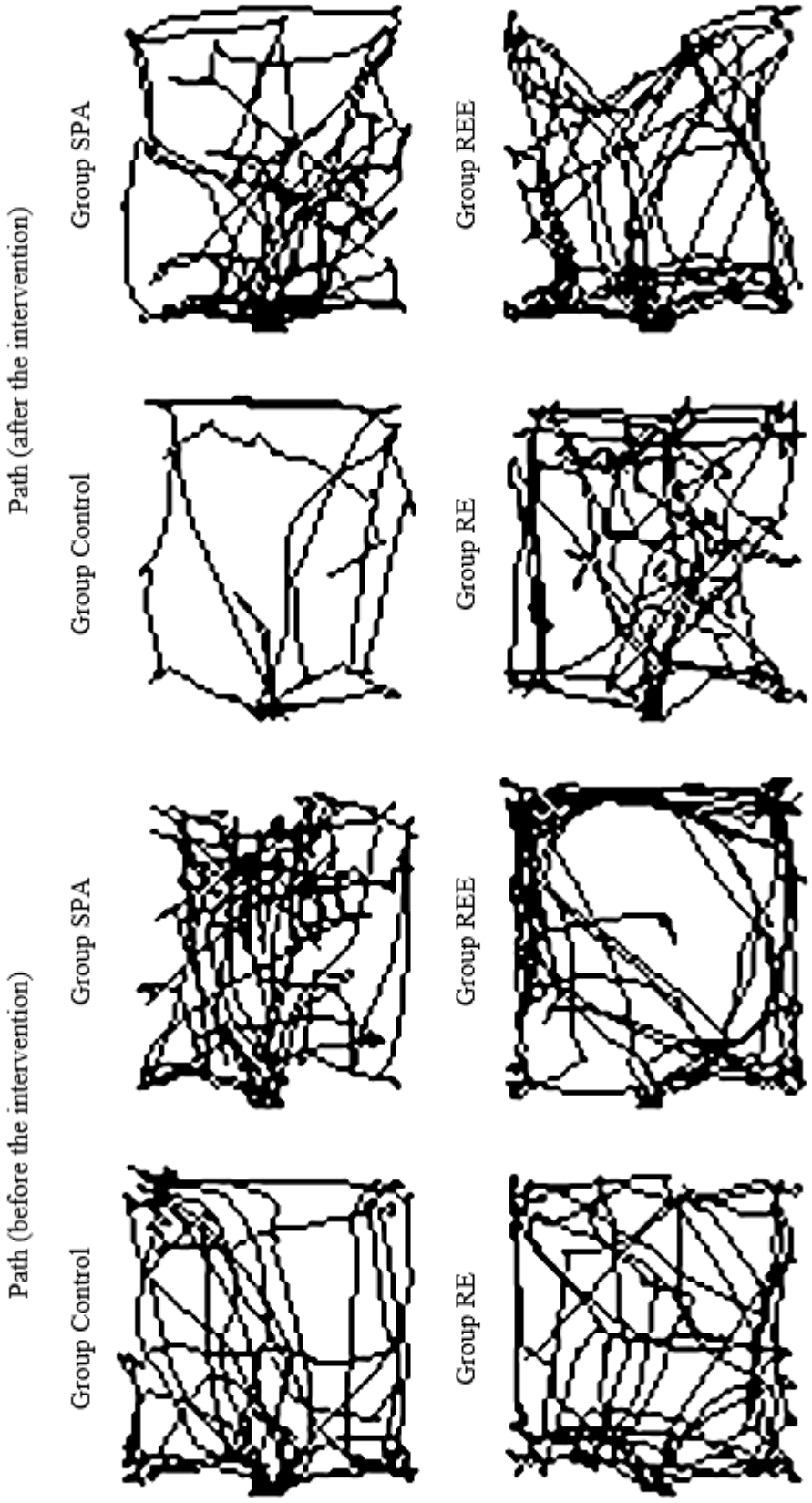


Image during the test
Group SPA
Group REE
Group Control
Group RE
Path (after intervention - respond acutely)
Group SPA
Group REE
Group Control
Group RE

Figure S8


Path (after the intervention)
Group SPA
Group REE
Group Control
Group RE
Path (before the intervention)
Group SPA
Group REE
Group Control
Group RE

3.3 Artigo Científico 2:

Effects of resistance training and physical activity on skeletal muscle and white adipose tissues, myokines and adipokines gene expression, cancer-related cachexia occurrence, and cancer-related survival of C57BL/6 mice bearing syngeneic cutaneous melanoma

Periódico Alvo: Jornal of Cachexia, Sarcopenia and Muscle

Effects of resistance training and physical activity on skeletal muscle and white adipose tissues, myokines and adipokines gene expression, cancer-related cachexia occurrence, and cancer-related survival of C57BL/6 mice bearing syngeneic cutaneous melanoma

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ABSTRACT

Background During tumor progression, might occur cancer-related cachexia (CRC), a paraneoplastic syndrome characterized as progressive, systemic physical consumption state of individual with cancer. It has been hypothesized that resistance training (RT) and physical activities (PA) can modulate molecular signaling pathways that influence skeletal muscle tissue (SMT) and white adipose tissue (WAT) in individuals with cancer. **Aim** We investigated the effects of RT and PA on plasma inflammatory biomarkers, on weight, strength, and cellularity of SMT and weight and cellularity of WAT, on myokines (*MYOG*, *FXOB32*, *IGF1*, *TRIM55*, and *TRIM63*) and adipokine (*PPARγ*) gene expression in controls and C57BL/6 mice bearing syngeneic cutaneous-melanoma model. **Material and Methods** Murine B16F10 cells were injected into flank of the fifty-eight female controls (non-tumor induction) and C57BL/6 mice bearing syngeneic tumor. CRC diagnosis was individually established for each animal using as parameter weight loss ≥ 10%. Mice were randomly distributed in 8 groups: G1 (non-tumor induction, sedentary mice, n = 5), G2 (non-tumor induction, RT, n = 5), G3 (non-tumor induction, SP, n = 5), G4 (tumor induction, sedentary mice, n = 15), G5 (RT earlier tumor induction, n = 16), G6 (RT earlier and after tumor induction, n = 16), G7 (RT after tumor induction, n = 13), and G8 (PA after tumor induction, n = 10). The consumption of water and food, body weight, and tumor size were daily measured. Albumin and C-reactive protein (CRP) serum levels were measured by enzyme immunoassays. SM strength, volume, and mass were assessed using a grip strength meter, a high-frequency ultrasound, and analytical balance, respectively. SM and WAT samples were collected and submitted to morphometric and gene expression analysis. Control and experimental mice were submitted to cancer-related survival (CRS) analysis. **Results** Our findings showed that mice bearing tumor submitted to RT and SPA exhibited increase of the muscle mass and strength. However, neither RT nor PA exhibited influence on myokines and adipokines expression in SMT and WAT, respectively, between control and mice bearing tumors. Moreover, it was not showed any difference for CRC and CRS between controls and mice bearing tumors submitted to RT and PA ($p > 0.005$). **Conclusion** Thus, experimental studies with other methodological models of physical exercise with C57BL/6 mice should be encouraged to elucidate the gaps left here.

Key-words: cancer-related cachexia, C57BL/6 mice, B16F10 mouse melanoma cell, resistance training, physical activity, cancer-related survival.

INTRODUCTION

Cancer-related cachexia (CRC) is defined as a multifactorial syndrome which occur an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional supports, and leads to progressive functional impairments in individuals with cancer [1]. CRC is a systemic pathological condition rather prevalent that starts sinceearly stage of tumor progression. Clinically, CRC is characterized by an involuntary, progressive physical consummation that mainly occurs due to a variable combination of involuntary reduced food intake (anorexia), sarcopenia, negative energy balance that occurs in adipose tissues, and a low-grade, chronic systemic inflammatory state [2, 3]. From the clinical point of view, individual with CRC has not been diagnosed readily, accurately assessed and successfully managed. Consequently, individuals with CRC usually exhibits association with poor outcomes, such as, poor quality of life, low tolerance and reduced response to standard antineoplastic therapies, and a shorter survival [4, 5]. To date, there has been no effective pharmacological and non-pharmacological therapy to prevent or treat CRC. It has been shown in some studies that enhancement of dietary supplementation and administration is not effective in reversing or delaying the occurrence of CRC in individuals with cancer [6, 7]. In parallel, it has been reported that a set of non-pharmacological approaches have been employed for the prevention and treatment of CRC [8, 9]. Among these

approaches, the practice of resistance training and physical activities have been reported [10]. Notably, the findings of these studies have presented contradictory results and, therefore, the relationship between activity and physical exercise and cachexia still needs to be established.

Resistance training (RT), also known as strength exercise, is characterized by the use of equipment or free weights that offer a mechanical load opposite to the movement of the body segment of individual, stimulating several neural and skeletal muscle physiological adaptations [11, 12]. A RT program aims to enhance the trainable physiological characteristics, such as muscle strength, hypertrophy, power, and endurance[11, 12]. The maintenance time of the tension, the speed of execution of the movement, the type of accumulative metabolic, among other situations, are possibilities of the variability of stimuli that promote morphological and functional adaptations in the corporal system [11-14]. The improvement of muscle strength primarily uses the anaerobic energy system through neural adaptations, characterized by increased muscle strength, improved intermuscular, and intramuscular coordination, and muscle hypertrophy adaptations, characterized by increase in type II muscle fibers density and volume or increase of molecular components related to contractile function of the myofibrils [11, 12]. It has been demonstrated that the performance of resistance exercises can positively modulate many chronic local or systemic diseases [15-22]. It has been showed that PA might exhibit modulatory roles, with beneficial activities, in a number of systemic and chronic inflammatory diseases [23, 24]. It has been hypothesized that both RT and PA approaches should prevent or even reverse the negative effects promoted by CRC in individuals with cancer. However, the influence of RT and PA in improving the prognosis of individuals with CRC is not still established. In to concerns to performing RT and PA by individuals with cancer, several factors might be taken into account in order to avoid unwelcome detrimental effects.

The aim of this study was to analyze the effects of different approaches of RT and PA on CRC occurrence and survival of C57BL/6 mice with syngeneic CRC model. Moreover, we investigated in control and mice that model if RT and PA approaches could influence skeletal muscle mass and strength of hindlimb. Moreover, we compared a set of cytomorphometrical parameters in muscle fibers and adipocytes in SM and WAT, respectively. Finally, we also assessed the expression of catabolic genes in SM and WAT.

MATERIAL AND METHODS

Reagents

The chemicals used for culture for murine B16F10 cells were high glucose Dulbecco's modified Eagle´s medium (DMEM, Merck, Darmstadt, GER), fetal bovine serum (FBS), 0.05% trypsine-EDTA, 0.03% L-

glutamine, 100 U/ml penicillin, 10 μg/ml streptomycin, 10 μg ciprofloxacin/ml, 0.5 μg/ml amphotericin B, lipofectamine 2000, fetal bovine serum (FBS). For gene expression assays were used 50 mM β-mercaptoethanol, Trizol reagent, RNA holder that were purchased from Merck (Darmstadt, GER).

Cell culture

Mouse B16-F10 cutaneous melanoma (CM) cell line (ATCC® CRL-6475™) was obtained from of the Laboratory of the Antitumor Substances Laboratory, Institute of Biological Sciences, Universidade Federal de Minas Gerais (UFMG). The B16-F10 cells were cultured in DMEM supplemented with 10% FBS (v/v) (EuroClone), 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin (Lonza, Basel, Switzerland) and 50 mM β-mercaptoethanol. The culture medium was filtered with a 0.22 μm filter and stored at -4° C. The pH of this culture medium was adjusted to 7.4. In use, the culture medium will be preheated at 37°C in a 5% CO_2-humidified incubator. After reaching a minimum of 90% cell confluence in the culture plates, the adhered cells werereleased from the plate after treatment with trypsin for 5 minutes, oven temperature of 37 ° C, and 5% CO_2. Soon after, the trypsin was inactivated with 10% FBS. The detached cells were centrifuged (1000 rpm), suspended in RPMI culture medium and the cell concentration measured using Neubauer's mirrored chamber (OG-200, Grid Optics, Hong Kong, CHI).

Ethical aspects

This study is study is characterized as experimental, prospective, analytical, with quantitative approach. Study design for *in vivo* assays were approved by a relevant ethics committee in animal well-being and experimentation of the Universidade Estadual de Montes Claros (protocol number: 131/2017), which follows the recommendations of the *Brazilian Code for Use of Laboratory Animals* (Law 11.794, 2008) [25].

Animals

Eighty-five C57BL/6 female mice, 10-12 weeks old, with about 20 ± 5 g of body weight were used. Mice were obtained from Animal Facility Center, Institute of Biological Sciences from UFMG, Belo Horizonte, Brazil, for experimental use in this study. Mice were maintained under controlled temperature (24 ± 1°C), light (12 h of light/12 h of darkness), relative air humidity (60-70%) and allowed free access to water and balanced food chow (Purina-Labina®, São Paulo, BRA). Animals were randomly housed in groups of 5-8 individuals in autoclavable polypropylene boxes, with dimensions of 414 x 344 x 168 mm, covered with galvanized steel, and containing

separators in stainless steel (Zootech, model ZT 375, Paraná, BRA). After one-week acclimatization on standard chow diet, 6 mice were euthanized for baseline analysis of target tissues investigated in this study. All animals were daily monitored in order to analyze the water and food consumption and body weight.

Syngeneic tumor model

Prior to subcutaneous inoculation in C57BL/6 mice for tumor induction of the syngeneic mouse CM model, the B16F10 cells were removed from culture flasks by adding 0.05% of trypsin solution, centrifuged, and ressuspended in sterile PBS, and then suspended in 50 μL of incomplete RPMI medium. C57BL/6 mice were subcutaneously inoculated with 5×10^5 cells/animal into the flank using a 1-ml tuberculin syringe (Hamilton Co., Reno, NV) with a 27-gauge hypodermic needle. Inoculation of this number of viable cells in the subcutaneous region is able to complete a mitotic cycle within 24 hours and to develop the tumor within 3-4 days. The control group was inoculated with 0.1 ml PBS only in the same anatomical region. All mice developed syngeneic CM model.

Diagnosis of the syngeneic CM-related cachexia

All mice were evaluated daily in to concern to water and food intake using an analytical balance and a plastic beaker with volume scale, respectively [26]. All mice were daily weighed in order to verify the body weight loss promoted by experimental tumor progression using an analytical balance. CM-related cachexia was established as soon as the mice bearing syngeneic CM model show a loss of *at least 5-10% of body weight* during tumor progression [27]. All mice were monitored daily for occurrence of others signs or symptoms of CRC, such as prolonged inactivity, dehydration, arched posture, occurrence of dry or color change of skin, coarse/rough coat, reddened eyes, and discharge of nasal secretion [28]. A linear regression equation was used to define the relationship between tumor volume and tumor weight. Measurements were taken on 12-day after tumor cell inoculation in a sample of animals (n = 12) in order to calculate the tumor weight throughout all experiment. Each day, one mouse was euthanized and the tumor volume (mm^3) and mass (g) were measured in order to obtain tumors with different volume [29]. 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 ($R^2 = 0.9892$). Once defined the tumor weigh measurement, these values were subtracted from body weight measurement of each animal per day.

Study groups

Mice were randomly distributed into eight groups, as follows: : i. control mice, no tumor induction, sedentary (G1, n= 5); ii. mice submitted to resistance training (RT), no tumor induction (G2, n = 5); iii. mice submitted to spontaneous physical activity (SPA), no tumor induction (G3, n = 5); iv. mice with tumor induction, sedentary (G4, n = 15); v. mice submitted of RT prior to tumor induction (G5, n = 16); vi. mice submitted to RT before and after tumor induction (G6, n =16); vii. mice submitted to RT, after 10 days of tumor induction (G7, n = 13); viii. mice submitted to SPA ,after 10 days of tumor induction (G8, n = 10).

Resistance training and spontaneous physical activities

Resistance training (RT) consisted in climbing movement of mice on the vertical ladder (1.1 × 0.18 m, spaced 2 cm between grid steps and 80 ° tilt) [17, 18] with electric stimulation (20 volts of intensity and 45 Hz of frequency was applied to the four legs of the animal) [30]. Animals performed 6 sets of 8 repetitions with intervals of 90 seconds between sets, with no external resistance added. Animals of the G3 and G8 groups underwent AF (Physical Activity) intervention, here in the study also called spontaneous physical activity (SPA), which occurred in a closed enriched environment (plastic box) with 60 cm in length, 30 cm in width and 45 cm in height, this environment was composed of seesaw, wheel, balls and tunnels[23, 30, 31]. In order to avoid compromising the experimental protocols, two moments of familiarization of the animals were performed before each of them. The research team performed pilot procedures for technical calibration.

Measures of skeletal muscle volume

Measurement of the skeletal muscle (SM) perimeter and volume from right hindlimb of C57BL/6 mice were assessed three times a week. SM perimeter was performed using a digital caliper, with sensitivity of 0.01 mm, CSX-B model (Mitutoyo, São Paulo, BRA). SM volume measurement was performed using a Doppler ultrasound imaging device (LOGIQ® e Pro, GE HealthCare Medical Systems Inc., Wauwatosa, WI, USA) with dynamic range of 258 dB and frame rate of 1449 frames per second. The electronic linear probe with 11.1-mm x 34.8 mm footprint, and bandwidth of 6.7-18 MHz imaging frequency was used (L8-18i-RS). Gain, frequency, focus position, and depth parameters were adjusted appropriately to make sure that the SM tissues (gastrocnemius, tibialis, and quadriceps) were displayed clearly on the screen, scanned transversely and longitudinally.

Sonographic images were stored in the internal hard disk of the device for subsequent analysis [32]. Soon after euthanasia of mice (G4, G7, and G8), the right hindlimb was removed and SM masses were separated from bone e assessed using an analytical balance [33].

Skeletal muscle strength analysis

An automatic dynamometer (Bonther®, São Paulo, BRA) was used to perform pre-limb grip strength analysis [30, 34, 35] . The assessment of muscle strength in all groups occurred 7 days before inoculation on the day of cancer induction and 10 and 15 days after. The mice were initially weighed, the meter restarted, choosing grams as unit / value scale, raising the animal by the tail to the height where both hind and hind limbs were at the same height as the bar, moved the animal horizontally to the bar until reaching the visually verified, tight and symmetrical grasp with the two hindlegs and hindlegs and lower limbs exerting a detectable resistance against the researcher's tug, gently pulled the animal away until its grasp was separated. The traction was at a constant speed and slow enough to allow the mouse to increase the resistance against it, to turn backward during the traction or to leave the bar without resistance. Sufficient time (at least one minute interval) between measurements was allowed to allow the animal to recover and avoid habit formation. The total force peak was measured and the average of three attempts was recorded. Normalized grip strength was obtained by dividing the value of muscle strength by the animal's body weight.

Plasma inflammatory biomarkers measurement

At the time of sacrifice of mice, peripheral blood from all mice was collected after an overnight fast. Serum albumin levels were measured using the bromocresol green dye-binding method with a Roche Modular DP analyzer device (Roche Diagnostics, Basel, SWI) used the Labmax plenno automation (LabTest Diagnóstica, Lagoa Santa, MG, BRA). High-sensitive C-reactive protein (CRP) level was determined with a highly sensitive latex reagent immunoassay [36].

Tissues samples analysis and histomorphometric studies

After euthanasia, the posterior limb SM (gastrocnemius), white adipose tissue (WAT) of the inguinal region were removed, weighed, duly fixed and stored. A fragment of the samples was embedded in optimal cut temperature compound (Tissue-Tek®, Sakura Finetek, USA), frozen in liquid nitrogen-cooled isopentane, stored at -80°C, and posteriorly cut into 10 µm thick cryosections with a cryostat (Microm HM525, Thermo Fisher

Scientific, Waltham, MA, USA). Another fresh fragment was kept in RNA holder for posterior gene expression assays. A third fragment was removed and placed in buffered 10% formalin solution overnight and embedded in paraffin and submitted to five-micron-thick sections using a Leica RM2125 (Leica Biosystems, IL, USA) microtome. SM, and WAT sections were stained with H&E and Masson´s Trichrome. Tissue sections were observed with microphotographs from these tissues were taken with an Olympus FSX100 microscope (Tokyo, JPN). Histomorphometrical analyses were performed using ImageJ software [37, 38].

Initially, microscopic image calibration was performed to recognize the sample size based on the number of pixels and magnification of the microscope. The pixel ratio was set to 1, the unit length in μm and the "Global" box was selected to maintain the settings of all subsequent image analyzes. To determine these parameters the image was taken with the same magnification of the images of interest [39]. Limit image adjustment was performed with the objective of providing defined areas consisting of membrane material and void space identified by black and white, respectively. After defining the areas of interest, the image was converted into a binary format to allow analysis. Each membrane was uniformly augmented to define the cell membrane and to identify the individual area of each cell. Using the "Measure and label macro" tool of ImageJ, the cells were defined by means of a yellow trace in their interior and by a unique number in the center [40]. Later the tool "Wand" was in charge of counting, being that the cells that touched the edge of the image were not counted. After the quantification and measurement of the areas, the recorded data were copied, followed by the statistical analysis [41].

RNA isolation, cDNA synthesis, and quantitative real time-polimerase chain reaction (qPCR) gene expression analysis

Total RNA was isolated using Trizol according to the manufacturer's protocol. Genomic DNA contamination was removed with DNase I (Deoxyribonuclease I). Reverse transcription reaction was performed using transcriptase reverse (M-MLV Reverse Transcriptase), OligodT primer, Random Primers and Rnase Out.. The RNA concentration was measured by UV absorbance. RNA samples with an A260/280 ratio between 1.8 to 2.0 were used. RT-PCR reactions were set up in duplicate using TaqMan gene assays, and amplification reaction was conducted on QuantStudio™ 6 Flex (Thermofisher). The amplification reactions were conducted according to the manufacturer's instructions with the aid of Taqman assays. Primers evaluated on muscle tissue samples were MYOG (Mm00446194_m1), IGF1 (Mm00439560_m1), FBXO32 (Mm00499523_m1), TRIM63 (Mm01185221_m1) and TRIM55 (Mm01292969_m1). GAPDH (Mm99999915_g1) and PPARG (Mm00440940_m1) were evaluated on adipose tissue samples. Internal template controls (GAPDH) run under the

same conditions. For each gene, the number of cycles required for exponential amplification was determined on 40 cycles. Gene expression levels were normalized to the level of GAPDH expression. Paired t-tests were conducted on relative quantity (RQ) values for each group to determine their significance.[42]. Gene expression levels were normalized to the level of GAPDH expression. Paired t-tests were conducted on relative quantity (RQ) values for each group to determine their significance.

Survival analysis

Control (G1, n = 10) and experimental G2, G3, G4, G5, G6, G7, and G8, n = 10, in each group) mice groups were submitted to follow-up for of 20 days at most after CRC diagnosis in order to compare overall cancer-related survival (CRS) between study groups. Kaplan-Meier survival curves were estimated for each event and the curves of the different groups were compared using the log-rank test. However, any mice were euthanized, as soon as, they become moribund, exhibiting severe impairment of bodily functions or behavior due to extensive necrosis, ulceration, and growth of the tumor mass.

Statistical analysis

All the collected data were inserted in an electronic database. Subsequently, the data were analyzed statistically in the SPSS software (Statistical Package for the Social Sciences) 20.0. All data are presented as mean ± S.D. The confidence level adopted in all analyzes was established in 95% ($p < 0.05$). Shapiro-Wilk tests were performed to verify normality. After this analysis, the paired Student's t-test and the ANOVA test were selected.

RESULTS

RT promoted increased skeletal muscle strength in C57BL/6 mice bearing CM.

Analysis of skeletal muscle (SM) strength in mice groups G1, G2 G3, G4, G5, G6, G7, and G8 occurred 7 days earlier tumor induction (baseline), and 10 and 15 days after tumor induction. Overall, all mice bearing tumor that were submitted to RT presented increases (analysis of delta values) for SM strength (G2 = 15.43 ± 15.11 g, G5 = 19 ± 27.73 g, G6 = 15 ± 14.89 g, and G7 = 27.5 ± 14.37 g). Additionally, mice submitted to SPA also gained (analysis of delta values) SM strength (G3 = 12.06 ± 5.59 g, and G8 = 2.50 ± 5.33 g). Mean values for absolute SM strength (Figure 1A) and variation of delta (Δ) values (Figure 1B), and inferential analysis comparing groups G1, G2, G3, and G4 are exhibited (Figure 1C). Paired Student's t test showed that mice not bearing tumor and submitted to RT (G2) and SPA (G3) exhibited gain of absolute and relative SM strength. On the other hand,

mice with tumor induction and sedentary showed decreased of SM strength (Figure 1A-B). However, only mice from G3 exhibited significant increasing of SM strength during interval of observation of study ($p = 0.008$) (Figure 1C). In mice with tumor induction, mean values for absolute SM strength (Figure 2A) and variation of delta (Δ) values (Figure 2B), and inferential analysis between groups G4, G5, G6, G7, and G8 are showed (Figure 2C). According to our findings, mice bearing tumor that were submitted to both RT and SPA exhibited gain of absolute and relative SM strength. However, mice that performed RT before and after (G6) and only after tumor induction (G7) exhibited significant increase of SM strength ($p < 0.01$, for both groups). Variation of the delta (Δ) values for SM strength showed significant difference between the mice groups G4 (no-tumor induction, sedentary) *vs.* G7 (RT after tumor induction) and G7 *vs.* G8 (PA after tumor induction) ($p = 0.005$ and $p = 0.019$, respectively).

RT promoted increased skeletal muscle volume in C57BL/6 mice bearing CM.

Ultrasonography images of the right hindlimb volume (mm^3) from C57BL/6 mice tumor bearing groups were gathered after tenth- and nineteenth-days post-tumor induction. Data were compared using paired Student's t-test. Mice from G4 exhibited a significant decreasing of the SM volume between examinations ($p = 0.001$). However, mice from G8 exhibited increase of the SM volume between examinations ($p = 0.000$). On the other hand, Bonferroni-correction after one-way ANOVA showed significant difference between for SM volume for mice groups G4 vs. G8 ($p = 0.024$), G7 vs. G8 ($p = 0.026$) (Figure 3).

RT and SPA promoted increased body weight in control but not in C57BL/6 mice bearing CM.

All mice had body weight, inguinal white adipose tissue (WAT), and quadriceps femoris SM tissue (SMT) weights assessed. On the first and seventh day of follow-up, the body weight from experimental mice were estimated discounting the tumor weight. The tumor weight was estimated using a linear regression equation according to formula: $y = 0.0006x + 0.0769$, which x is tumor volume and y represents tumor mass, with value of $R^2 = 0.9848$ (positive co-operation). Body weight measurements were G1 (21 ± 1.41 g), G2 (20.9 ± 1.13 g), G3 (21.93 ± 1.27 g), and G4 (17.86 ± 0.61g). Paired Student´s t test showed that mice bearing CM exhibited higher body weight loss compared to mice sedentary ($p = 0.022$), submitted to RT ($p = 0.027$), and submitted to SPA ($p = 0.008$). Mean values for SMT weight for mice groups were, as follows G1 (0.164 ± 0.06 g), G2 (0.294 ± 0.05 g), G3 (0.163 ± 0.04 g), and G4 (0.120 ± 0.01 g). Mean values for WAT weight for mice groups were, as follows G1 (0.156 ± 0.06 g), G2 (0.164 ± 0.06), G3 0.313 ± 0.14 g), and G4 (0.120 ± 0.18 g).

RT promoted higher skeletal muscle fiber area

We investigated the number (unity), nuclei count (unity), and SM fiber area (μm²) from gastrocnemius in control and mice bearing tumor submitted to RT and PA. Mice submitted to RT exhibited higher skeletal muscle fiber area compared to sedentary mice ($p = 0.003$), mice submitted to SPA ($p = 0.018$), and sedentary mice bearing tumor ($p = 0.043$) (Figure 6A). Mice submitted to RT earlier cancer-related cachexia (CRC) showed higher muscle fiber area compared to mice submitted to RT earlier and after CRC ($p = 0.013$) (Figure 6B).

Neither RT nor PA promoted changes in adipocyte number and cell area in WAT from control and C57BL/6 mice bearing tumor

We investigated the number (unity) and of adipocytes area (μm^2) from inguinal WAT from control (G1, G2, and G3) (Figure 7A) and mice bearing tumor (G4, G5, G6, G7, and G8) sedentary or submitted to RT and PA (Figure 7B). Comparisons between control and mice bearing tumor groups did not show significant differences between the groups ($p > 0.05$). Representative microscopical tissue images of the inguinal WAT of both control and mice groups (magnification: 200X) (Figure 7C).

Neither RT nor PA modulate expression of the myokines and adipokines related to cancer-related cachexia (CRC) in controls and mice bearing tumor

We selected six myokines genes (*MYOG*, *FXOB32*, *IGF1*, *TRIM55*, and *TRIM63*) and two adipokines genes (*PPAR-γ*) related to modulation of signaling pathways that modulate CRC pathogenesis. qPCR was performed in order to investigate relative gene expression of the selected myokines and adipokines in quadriceps femoral SMT and inguinal WAT from controls and C57BL/6 mice bearing tumor. Overall, it was not noted any difference for both myokines and adipokines relative expression between control and sedentary mice bearing tumor (G4), or submitted to RT (G7) or PA (G8) ($p > 0.05$). A significant difference for *TRIM55* expression was found between G1 *vs*. G7 groups ($p = 0.014$) (Figure 8).

Neither RT nor PA influenced CRC occurrence and CRS in C57BL/6 mice bearing tumor.

Kaplan-Meier curves represent the time of diagnosis for cancer-related cachexia (CRC) and overall cancer-related survival (CRS) of C57BL/6 mice bearing tumor (Figures 9 and 10, respectively). Final body weight measurement was corrected with subtraction of the tumor weight. Mean time for CRC diagnosis occurred for 7 ±

0.89 days for mice from G4, 7.25 ± 0.78 days for mice from G5, 5.41 ± 0.90 days for mice from G6, 6.81 ± 0.97 days for mice from G7, 6.60 ± 1.01 days for mice from G8.

Log-Rank test not showed significant difference between study groups ($p > 0.05$) (Figure 9). Mice bearing tumor were monitored daily during follow-up of 20 days. Mean time for CRS diagnosis occurred for 19.60 ± 0.26 days for mice from G4, 19.06 ± 0.41 days for mice from G5, 18.81 ± 0.60 days for mice from G6, 19.18 ± 0.38 days for mice from G7, 18.10 ± 0.62 days for mice from G8. Log-Rank test showed no difference for CRS between study groups ($p > 0.05$) (Figure 10).

DISCUSSION

According to our findings mice bearing tumor submitted to RT and SPA exhibited increase of the muscle mass and strength. However, neither RT nor PA exhibited influence on myokines and adipokines expression in SMT and WAT, respectively, between control and mice bearing tumors. Moreover, it was not showed any difference for CRC and CRS between controls and mice bearing tumors submitted to RT and PA.

The significant increase in muscle strength in the groups (G3, G6 and G7) and the expressive mean delta value (Δ) of the G2 group (15.43 ± 15.11) showed the neural adaptation in the models proposed in this study, where the overload of this chronic process imposed on the animals possibly resulted in adaptations in the progression of intramuscular and intermuscular coordination, efficiency and relaxation of contraction (co-activation) [11, 43], providing the increase of muscular strength even in the animals affected by CRC. It is important to point out that in all RT and SPA models there is an increase in muscle strength despite CRC. In groups G1 and G4 there was a decrease in muscle strength, a situation that also characterizes the cachexia.

In the analysis of the body composition, two situations were performed. The first one was an ultrasound evaluation of the total volume of the right hind limb, where G4, G7 and G8 groups were selected. The evaluation performed after cachexia showed that RT provided a significant increase when compared the delta value (Δ). The hypertrophic process apparently may have occurred due to resistance training [11, 43-45], a fact that cannot be affirmed, because in this evaluation, the calculated limb volume was the sum of the structures of muscle tissue, adipose tissue and bone tissue. However, the area of muscle fiber increased, therefore showing a perspective of hypertrophy.

In the second analysis of body composition, the evaluation was through direct weighing after dissection of the animals, the results generally show that the animals that had some type of treatment with RT or SPA have relevant results in the maintenance of body weight in the groups G2, G3, G7 and G8 have satisfactory weights of

the quadriceps femoris muscle. In the histomorphometric analysis of the muscle tissue, we also noticed that the G2 and G6 groups had the highest mean value of the muscle fiber area in the gastrocnemius muscle, showing that the animals that had a longer resistance training period presented muscle hypertrophy. It is noteworthy that the muscle fiber area of G6 is significantly larger than that of G7.

Results above can be explained as a function of the gain of the active muscular structures, this is attributed to the interventions with a longer training time, where they possibly the imposed overload may have provided a reduction of the systematic inflammation, decrease the oxidative stress, GLUT-4 (glucose transporter type 4) and increased protein synthesis via the IGF-I/Akt /mTOR pathway [14, 43, 46-51].

In the expression genes of interest in the muscle tissue of the femoral quadriceps, specific groups (G1, G4, G7 and G8) were analyzed, and the significant result occurred in the expression of TRIM55 between G7 vs. G7. G4, where the G7 group has a larger expression. Although IGF-1 expression is higher in G7, this group undergoes an antagonistic process with the expressive increase of the expression of the genes TRIM 55 (MuRF 2), TREM 63 (MuRF 1) and FBXO32, showing that the catabolic process is more pronounced than the anabolism in the muscle tissue analyzed. Expression of MuRF 1, MuRF 2 and FBXO 32 are associated with muscle atrophy [52-56]. The G8 group does not have this same expression, it presents results with a greater anabolic balance when compared with the other groups, showing that the optimized overload can potentiate better results in the muscular structure [57-59].

When there is no control of the total workload during the training process, overtraining may occur, this syndrome is responsible for the decrease in performance [58, 60]. The consequence of this state is the increase of the hormone cortisol at rest that consequently contributes to the process of decreasing lean mass (muscle tissue) [58, 60, 61]. This insufficient rest period results in a systemic inflammatory process that potentiates the reduction of lean mass [61, 62]. Although the literature deals largely with athletes regarding this syndrome, it is shown the possibility of involvement of this syndrome in aging or in some pathologies such as cancer and Parkinson's disease, since the oxidative stress already increased in these situations, seems to be involved in muscle fatigue and can lead to overtraining [63].

The histomorphometry of white adipose tissue and expression of the PPARγ gene showed no significant difference between the groups. However, it is noteworthy that the expression of PPARγ in G1 and G7 is lower. The RT proposed in this study, possibly leveraged by overtraining, may have provided lipid peroxidation. It is the process by which free radicals capture electrons from lipids in cell membranes, a fact that in cachexia is observed with increased lipolysis [24, 64-66]. Exercise has the ability to attenuate TNF-α action through increases in

circulating anti-inflammatory cytokines, a situation that is permitted by the increase in IL-10 in exercise that has a greater aerobic characterization [24, 66, 67], thus apparently the SPA provided a better expression of PPARγ. The SPA that was organized in this experimental model, provided neuromuscular and cardiorespiratory activities in the same environment, a fact that may have contributed to the results found here.

Regarding the diagnosis of cachexia and the survival of the C57BL/6 mice, our findings showed that TR and SPA did not provide longer time for cachexia affection and greater overall survival. As mentioned earlier, cutaneous melanoma is a type of cancer with a great aggressiveness, added to cachexia, becomes an aggravating factor for survival [27, 68-71].

FINAL CONSIDERATIONS AND CONCLUSIONS

The RT and SPA interventions contribute to increase muscle strength. RT and SPA showed improvement in body composition and histomorphometry, but better RT results after the cachectic framework were still insufficient, since we believe that the load control should be modified to better yield these variables, a fact that was evidenced in the genetic expression. Regarding survival, our findings showed that TR and SPA did not provide greater overall survival. Thus, experimental studies with other methodological models of physical exercise with C57BL/6 mice should be encouraged to elucidate the gaps left here.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could influence the impartiality of the research reported.

ACKNOWLEDGEMENTS

We would like to thank the Foundation for Research Support of the Minas Gerais State (FAPEMIG; grant numbers: PPM-00029-17 and DEG-00010-16); National Council for Scientific and Technological Development (CNPq, grant number: 150038/2017-8 and 430759/2016-9); and the Coordination of Improvement of Higher Education Personnel (CAPES) for the support to carry out this study. AMB De-Paula is research productivity fellow of the CNPq.

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Figures

Figure 1. Analysis of the skeletal muscle strength (SM) of the anterior limbs of the G1 groups (control, without tumor induction, sedentary), G2 (without tumor induction, RT), G3 (tumor induction, sedentary) occurred on the seventh day before tumor induction, and tenth and fifteenth days after tumor induction in C57BL/6 mice. Mean values for absolute strength SM (A) variation of delta values (Δ) for strength SM (B), and inferential analysis for each study group for each group are displayed (C). Bonferroni-correction after one-way ANOVA showed no significant difference between groups of mice. However, the paired Student's t test showed that G3 mice exhibited an increase in SM strength between the first and last exam ($p = 0.008$).

Figure 2. Analysis of skeletal muscle (SM) strength in mice groups G4 (tumor induction, sedentary), G5 (RT before cancer-related cachexia (CRC) diagnosis), G6 (RT before and after CRC diagnosis), G7 (RT after CRC diagnosis), and G8 (PA after CRC diagnosis) occurred 7 days earlier tumor induction, and 10 and 15 days after tumor induction in C57BL/6 mice. Mean values for of absolute SM strength (AMS) (A), variation of delta (Δ) values for SM of muscle strength (B), and inferential analysis for each study mice group are exhibited (C). Bonferroni-correction after one-way ANOVA showed significant difference between SM strength for mice groups G4 *vs*. G7 ($p = 0.005$), G7 *vs*. G8 ($p = 0.019$). Paired Student's t test showed significant differences between SM strength in mice from G6 and G7 exhibited an increase in SM strength between first and last examination ($p = 0.000$, for both groups).

Figure 3. Analysis of the skeletal muscle (SM) volume (ml) from right hindlimbof C57BL/6 mice bearing CM in tenth and nineteenth days after tumor induction. Ultrasonography images of G4 (tumor induction, sedentary), G7 (RT after tumor induction), and G8 (PA after tumor induction) groups Means values for SM volumetric assessments were compared using paired Student's t-test. Mice from G4 exhibited a significant decreasing of the SM volume between examinations ($p = 0.001$). Contrary, mice from G8 exhibited increase of the SM volume between examinations ($p = 0.000$). In the other hand, Bonferroni-correction after one-way ANOVA showed significant difference between for SM volume for mice groups G4 *vs*. G8 ($p = 0.024$), G7 *vs*. G8 ($p = 0.026$).

Figure 4. Analysis of body, inguinal white adipose tissue (WAT), and femoral quadriceps skeletal muscle tissues (SMT) weight assessments in control (sedentary [G1], resistance training [G2], and physical activities [G3]) and sedentary C57BL/6 mice bearing tumor (G4). Tumor weight was considered when weighing the body weight (g) of mice from G4 group. Means of groups were compared using analysis of variance (ANOVA) Bonferroni correction. Notably, sedentary mice bearing tumor exhibited lower body weight compared to controls (sedentary, RT, and PA groups). Comparison between controls and sedentary mice bearing tumor group for WAT weight did not exhibit significant differences. However, it was noted a significant decrease for SMT weight in sedentary mice bearing tumor compared to mice submitted to RT ($p = 0.018$). Mice submitted to RT showed higher SMT weight compared to sedentary mice ($p = 0.017$) and mice submitted to PA ($p = 0.019$).

Figure 5. Analysis of body weight, white adipose tissue (WAT), and skeletal muscle tissues tissue (SMT) assessments in C57BL/6 mice bearing tumor (G4, G5, G6, G7, and G8 groups). Tumor weight was considered when weighing the body weight (g) of the G4 group animals. Means values of the groups were compared using analysis of variance (ANOVA) Bonferroni correction. Mice submitted to PA exhibited higher SMT weight compared to sedentary mice ($p = 0.008$), and mice submitted to RT earlier and after ($p = 0.000$) and after tumor induction ($p = 0.002$) (A). Comparison between mice bearing tumor groups for WAT weight did not exhibit significant differences. However, it was noted significant increase for SMT weight in mice bearing tumor submitted PA compared to sedentary mice bearing tumor ($p = 0.008$), mice submitted to RT earlier ($p = 0.000$), earlier and after ($p = 0.000$), and after cancer-related cachexia (CRC) (0.002) mice groups. Mice submitted to RT showed higher SMT weight compared to sedentary mice ($p = 0.017$) and mice submitted to PA ($p = 0.019$).

Figure 6. Analysis of the number (unity) and skeletal muscle fibers area (μm²) of the gastrocnemius muscle in controls and mice bearing tumor groups. Cytomorphometrical analysis was performed using ImageJ software. Means values of the groups were compared using analysis of variance (ANOVA) Bonferroni correction. Mice submitted to RT exhibited higher skeletal muscle fiber area compared to sedentary mice ($p = 0.003$), mice submitted to PA ($p = 0.018$), and sedentary mice bearing tumor ($p = 0.043$) (A). Mice submitted to RT earlier cancer-related cachexia (CRC) showed higher muscle fiber area compared to mice submitted to RT earlier and after CRC ($p = 0.013$). Representative microscopical tissue images of the gastrocnemius muscle from both control and mice bearing tumor groups (stain: Masson´s trichrome, magnification: x200) (B).

Figure 7. Effect of RT and PA number (unity) and adipocytes area (μm²) from inguinal white adipose tissue (WAT) in controls (G1, G2, and G3) and C57BL/6 mice bearing tumor (G4, G5, G6, G7, and G8).

Cytomorphometrical analysis was performed using ImageJ software. Means values of the groups were compared using analysis of variance (ANOVA) Bonferroni correction. Comparisons between control and mice bearing tumor groups did not show significant differences between the groups ($p > 0.05$). Representative microscopical tissue images of the gastrocnemius muscle of both control and experimental mice groups (stain: H&E, magnification: 200X).

Figure 8. Effects of RT and PA on relative gene expression of selected myokines (*MYOG, FXOB32, IGF1, IGF2, TRIM55, and TRIM63*) and adipokines (*PPARγ and SREBF1*) in SMT (femoral quadriceps) and WAT (inguinal), respectively, of controls and mice bearing tumor groups. A significant difference was only found between *TRIM55* gene expression sedentary mice and mice bearing tumor submitted to RT after cancer-related cachexia (CRC) ($p = 0.014$).

Figure 9. Occurrence of cancer-related cachexia (CRC) in C57BL/6 mice bearing syngeneic cutaneous melanoma (CM). Mice groups (G5, G6, and G7) were submitted to resistance training (RT), submitted to physical activities (PA, G8), or categorized as sedentary mice bearing tumor (G4). Body weight measurement was corrected with subtraction of tumor weight. Data from mice bearing tumor groups were compared using Log-rank test and curves were represented by Kaplan-Meier plots. It was not showed any significant difference for CRC occurrence between all study mice groups ($p > 0.05$).

Figure 10. Analysis of cancer-related survival (CRS) in C57BL/6 mice bearing syngeneic cutaneous melanoma (CM). Mice groups (G5, G6, and G7) were submitted to in resistance training (RT), submitted to physical activities (PA, G8), or categorized as sedentary mice bearing tumor (G4). Data from mice bearing tumor groups were compared using Log-rank test and curves were represented by Kaplan-Meier plots. It was not showed any significant difference for CRS occurrence between all study mice groups ($p > 0.05$).

Figure 1

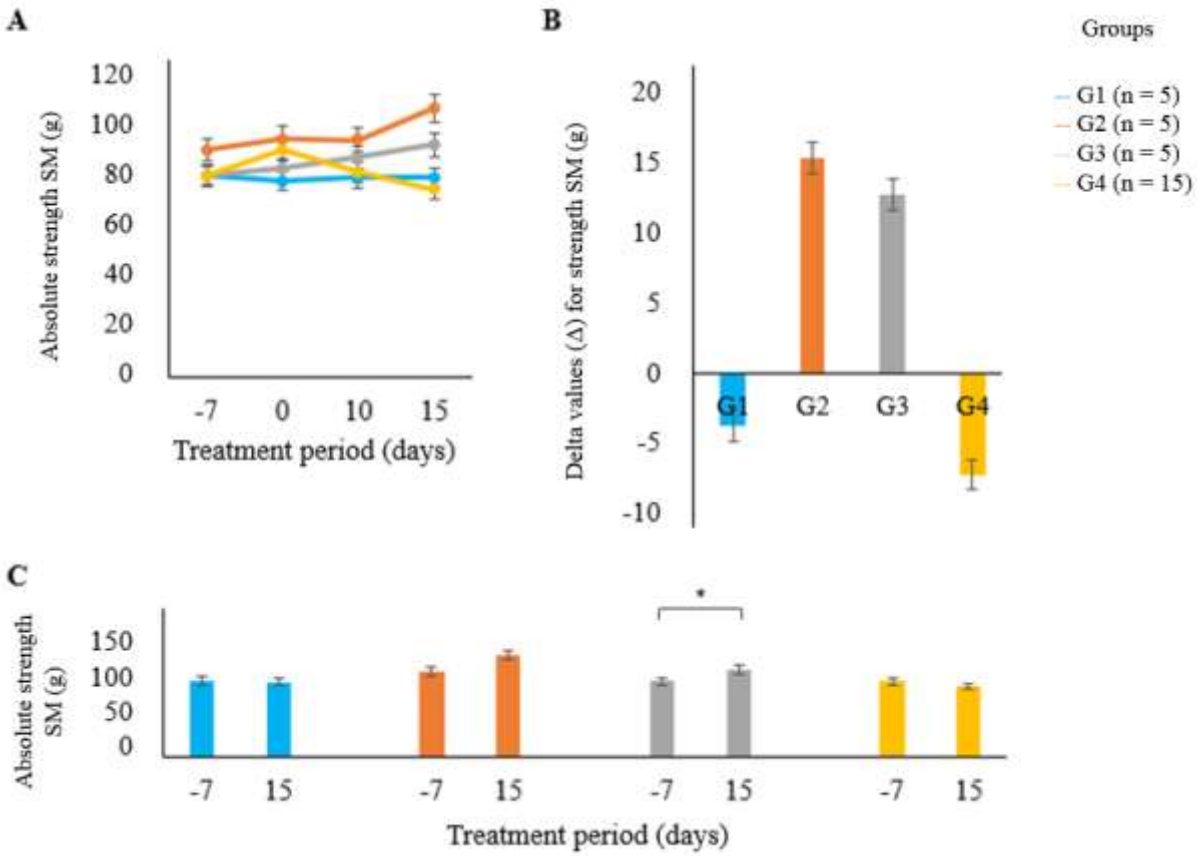

A
Absolute strength SM (g)
120
100
80
60
40
20
0
-7
0
10
15
Treatment period (days)
B
Delta values (Δ) for strength SM (g)
20
15
10
5
0
-5
-10
G1
G2
G3
G4
Groups
G1 (n = 5)
G2 (n = 5)
G3 (n = 5)
G4 (n = 15)
C
Absolute strength SM (g)
150
100
50
0
*
-7
15
-7
15
-7
15
-7
15
Treatment period (days)

Figure 2

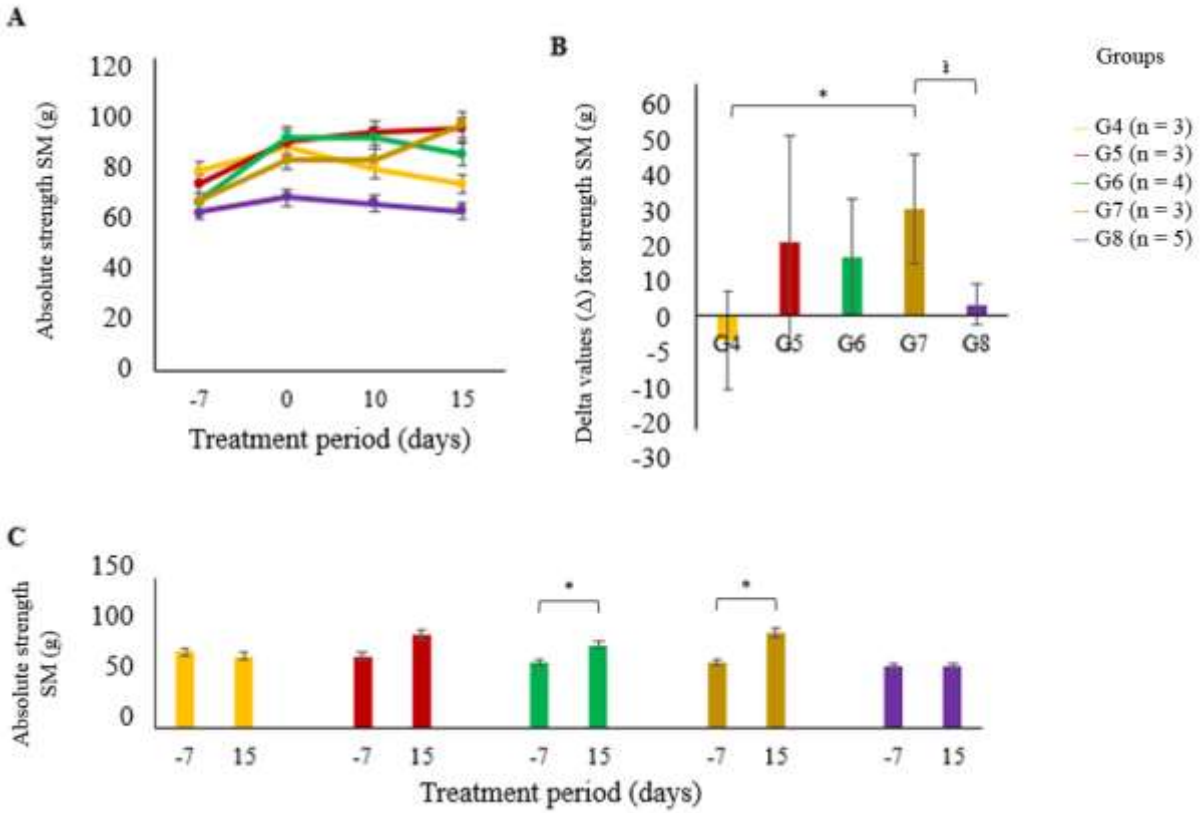

A
Absolute strength SM (g)
120
100
80
60
40
20
0
-7
0
10
15
Treatment period (days)
B
Delta values (Δ) for strength SM (g)
60
50
40
30
20
10
0
-5
-10
-20
-30
*
1
G4
G5
G6
G7
G8
Groups
G4 (n = 3)
G5 (n = 3)
G6 (n = 4)
G7 (n = 3)
G8 (n = 5)
C
Absolute strength SM (g)
150
100
50
0
*
*
-7
15
-7
15
-7
15
-7
15
-7
15
Treatment period (days)

Figure 3

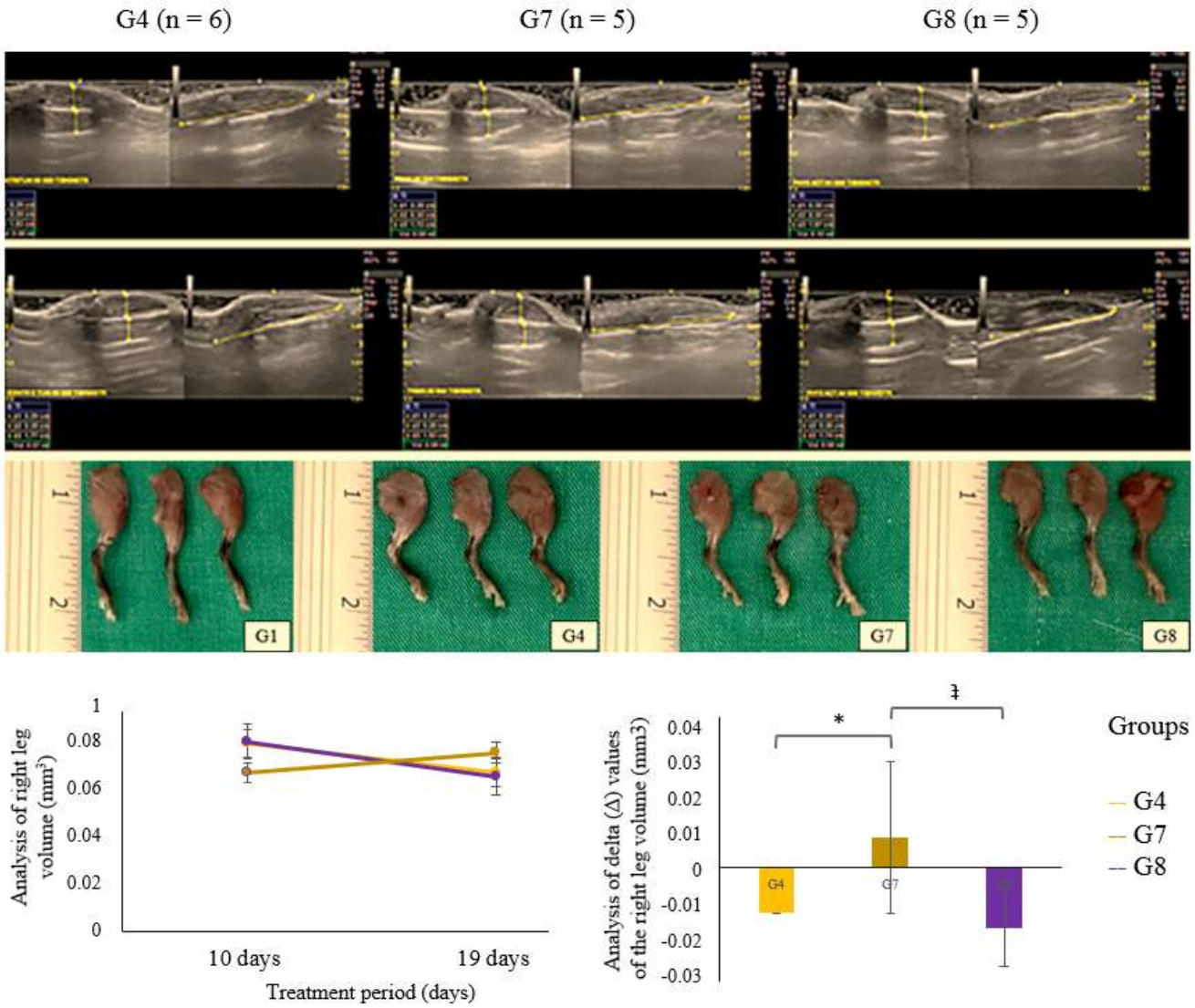

G4 (n = 6)
G7 (n = 5)
G8 (n = 5)
G1
G4
G7
G8
Analysis of right leg volume (mm³)
1
0.08
0.06
0.04
0.02
0
10 days
19 days
Treatment period (days)
Analysis of delta (Δ) values of the right leg volume (mm3)
0.04
0.03
0.02
0.01
0
-0.01
-0.02
-0.03
*
ǂ
G4
G7
Groups
G4
G7
G8

Figure 4

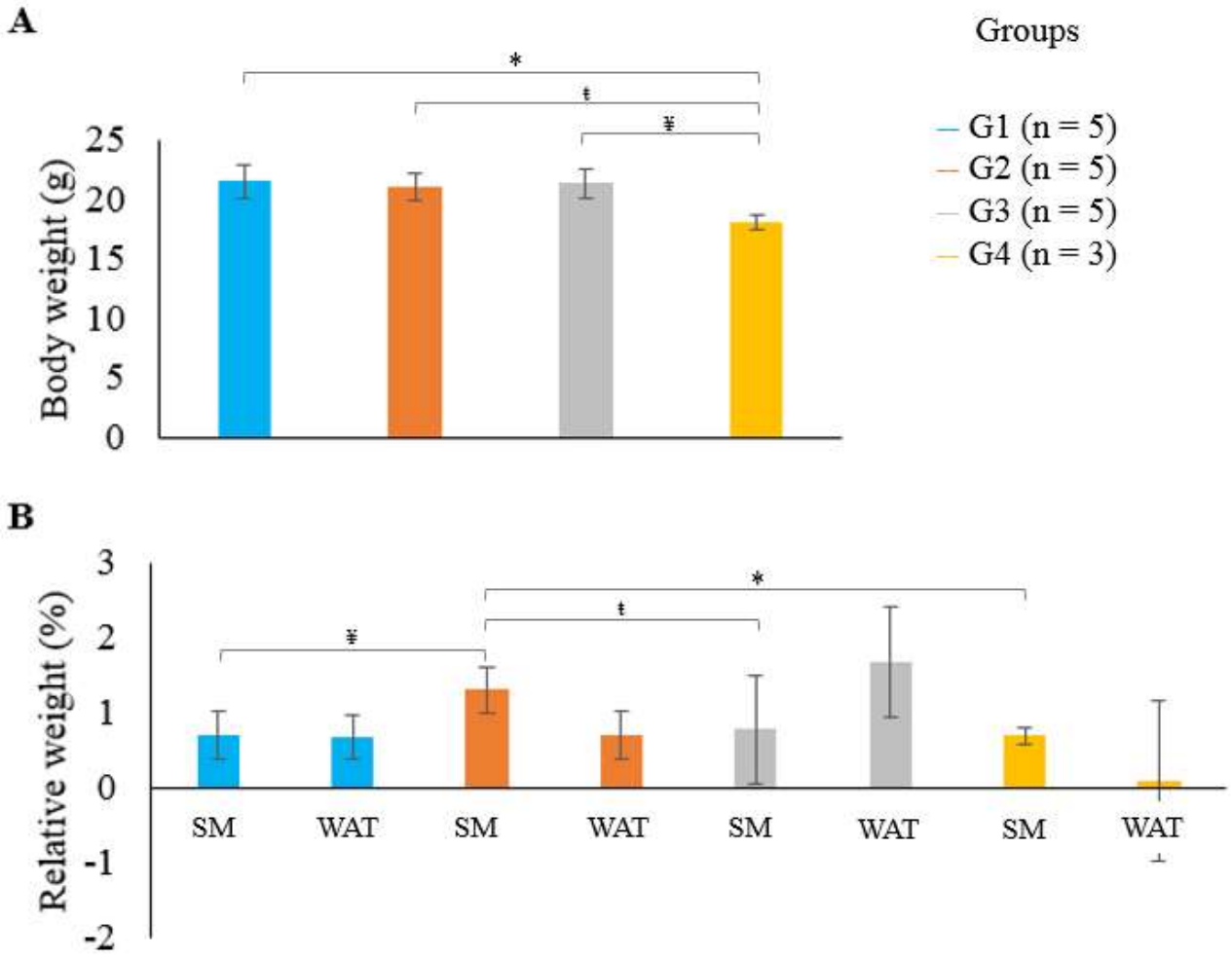

A
Groups
G1 (n = 5)
G2 (n = 5)
G3 (n = 5)
G4 (n = 3)
Body weight (g)
25
20
15
10
5
0
*
ŧ
¥
B
Relative weight (%)
3
2
1
0
-1
-2
SM
WAT
SM
WAT
SM
WAT
SM
WAT

Figure 5

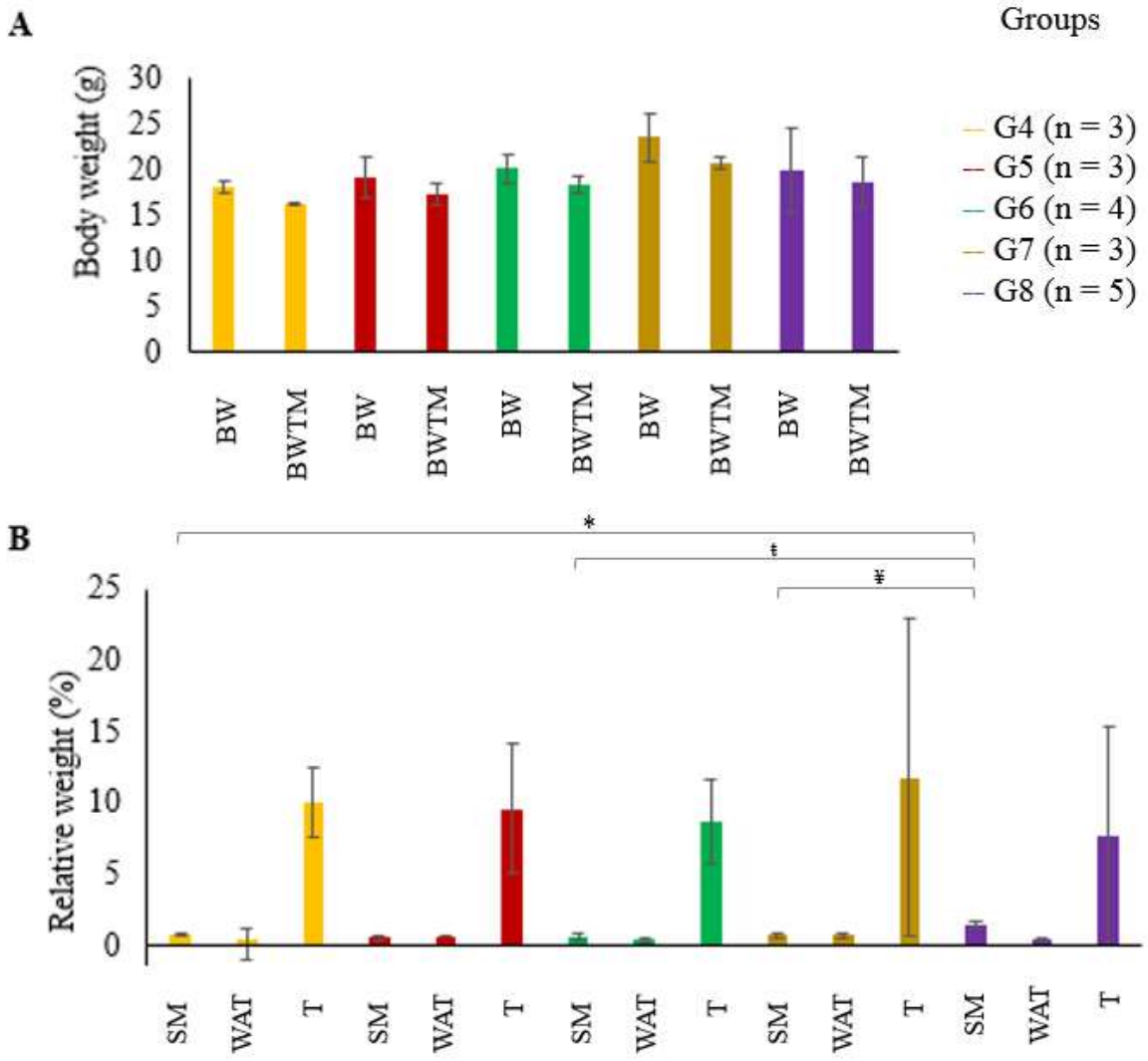

A
Groups
G4 (n = 3)
G5 (n = 3)
G6 (n = 4)
G7 (n = 3)
G8 (n = 5)
Body weight (g)
30
25
20
15
10
5
0
BW
BWTM
B
Relative weight (%)
25
20
15
10
5
0
SM
WAT
T
*
ǂ
ǂ

Figure 6

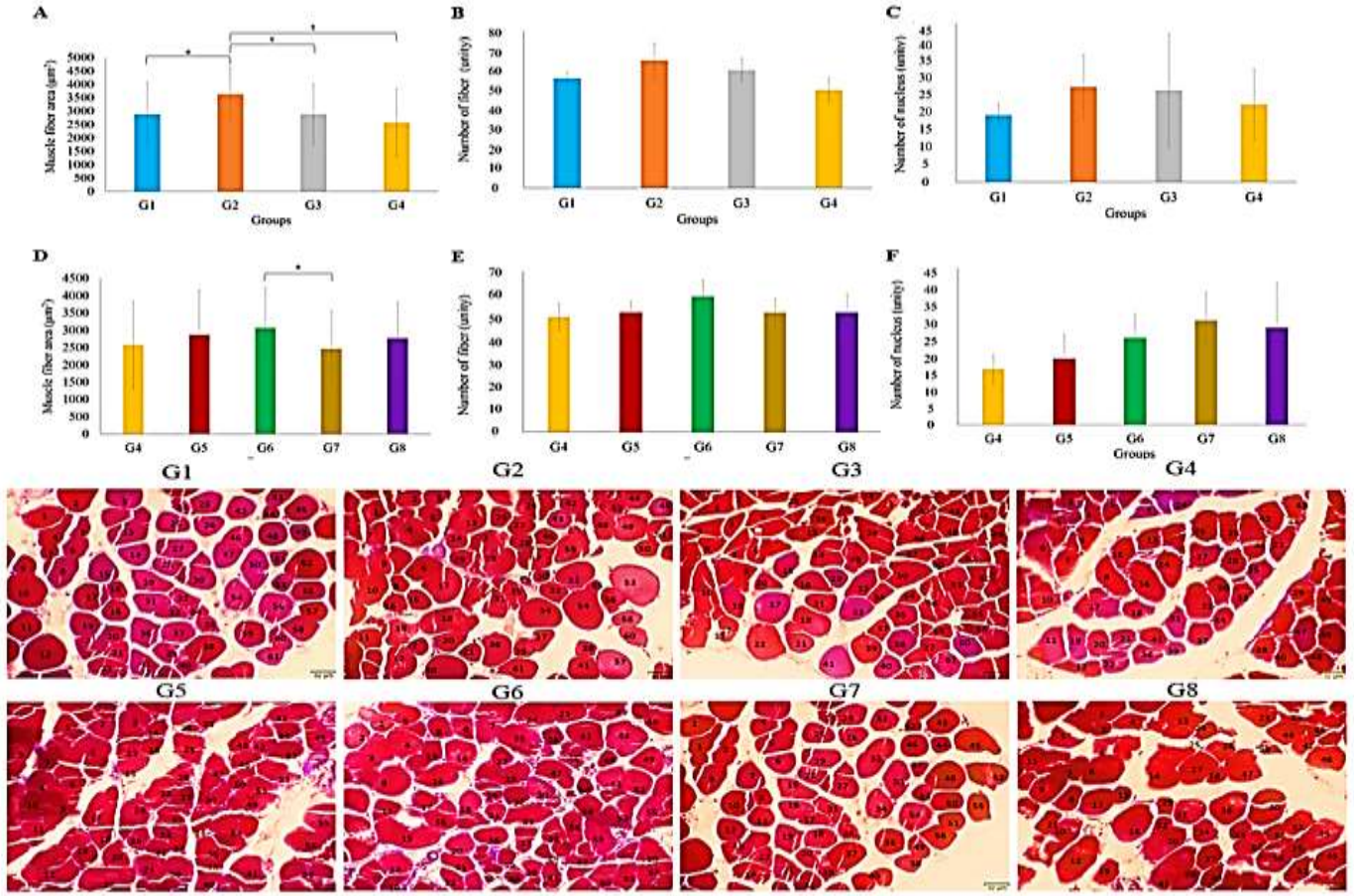

A
Muscle fiber area (μm²)
Groups
G1
G2
G3
G4
B
Number of fiber (unity)
C
Number of nucleus (unity)
D
E
F
Number of nucleus (unity)
G4
G5
G6
G7
G8
G1
G2
G3
G4
G5
G6
G7
G8

Figure 7

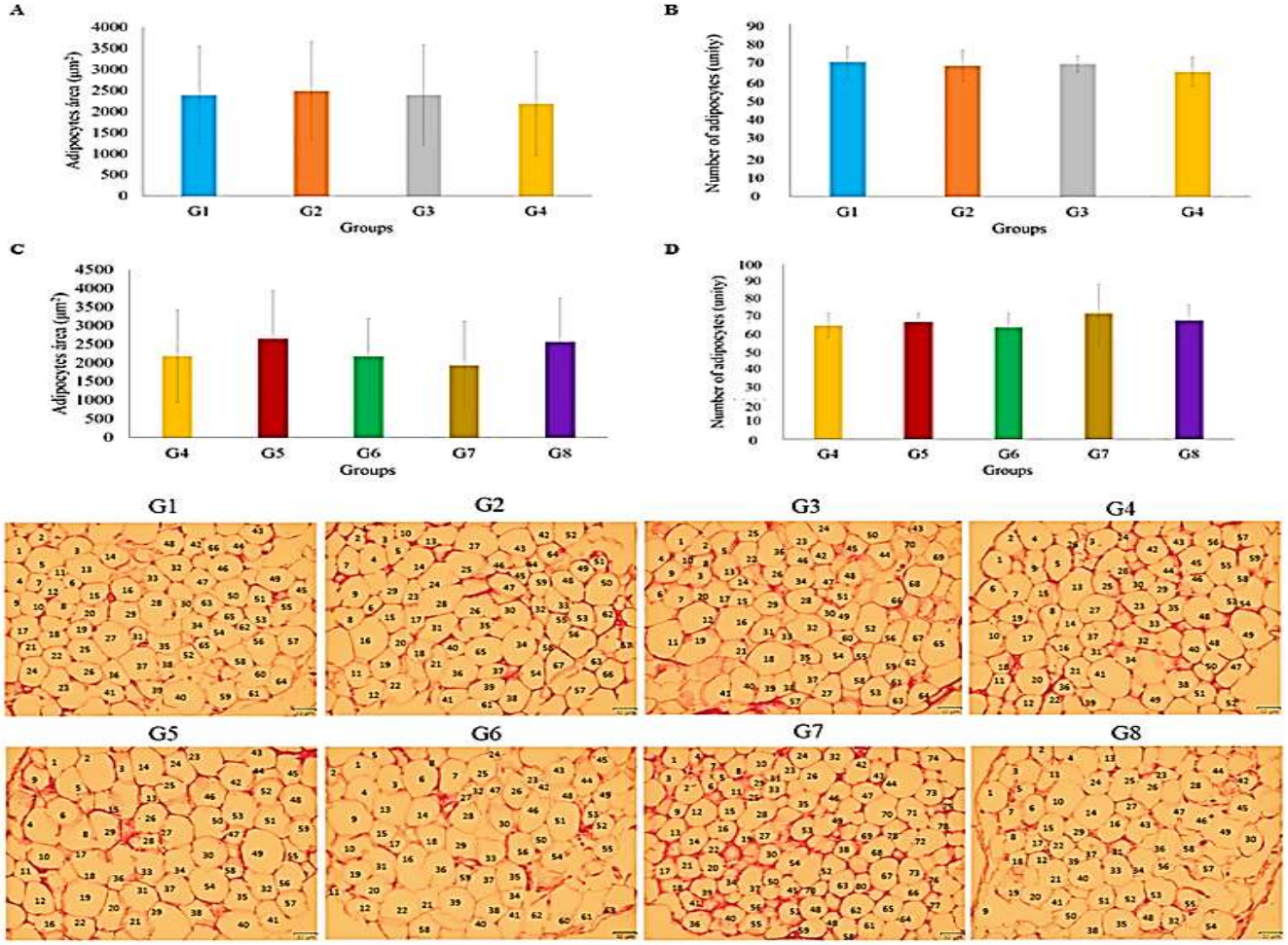

A
Adipocytes área (µm²)
4000
3500
3000
2500
2000
1500
1000
500
0
G1
G2
G3
G4
Groups
B
Number of adipocytes (unity)
90
80
70
60
50
40
30
20
10
0
G1
G2
G3
G4
Groups
C
Adipocytes área (µm²)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
G4
G5
G6
G7
G8
Groups
D
Number of adipocytes (unity)
100
90
80
70
60
50
40
30
20
10
0
G4
G5
G6
G7
G8
Groups
G1
G2
G3
G4
G5
G6
G7
G8

Figure 8

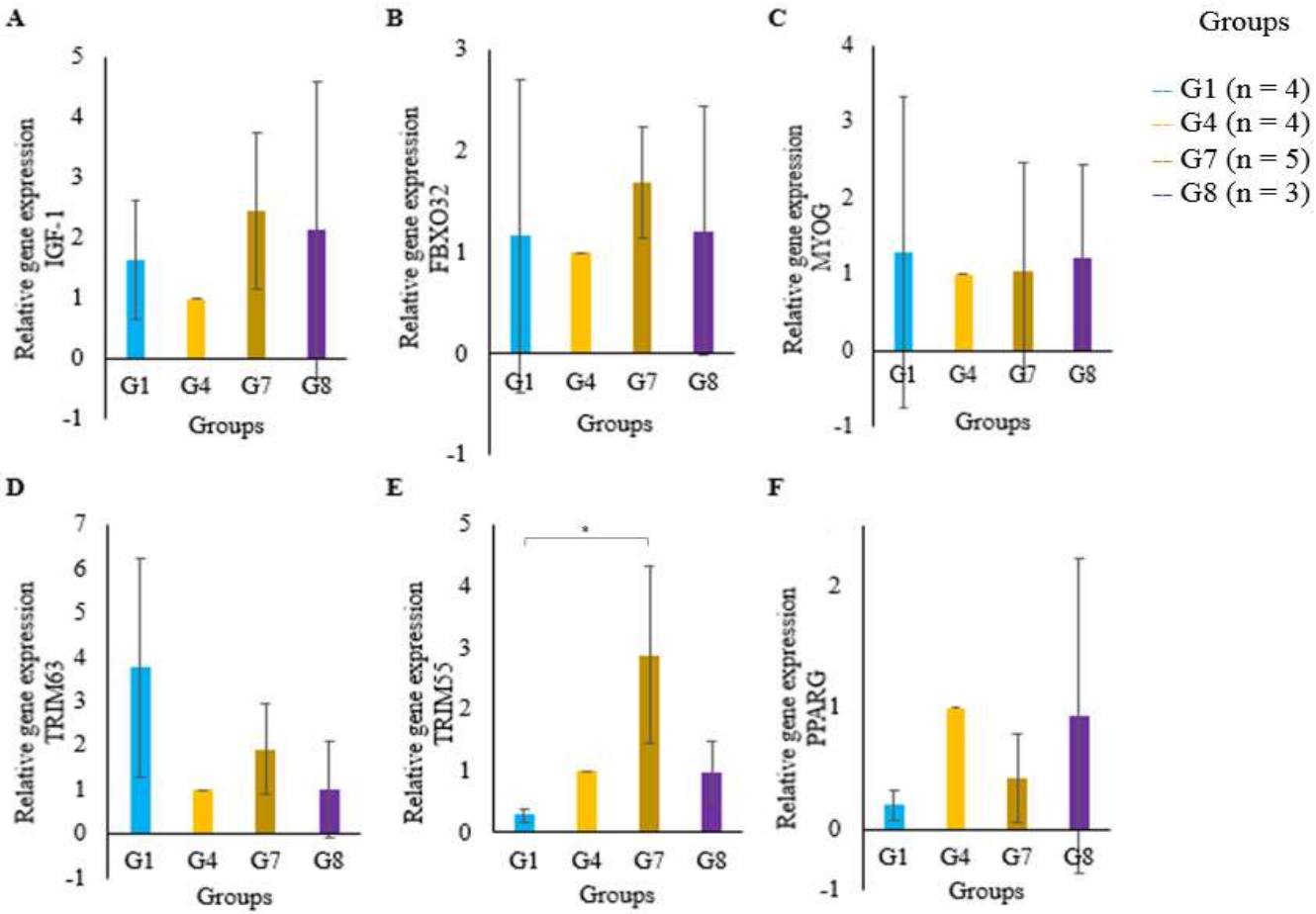

A
Relative gene expression IGF-1
5
4
3
2
1
0
-1
G1
G4
G7
G8
Groups
B
Relative gene expression FBXO32
3
2
1
0
-1
G1
G4
G7
G8
Groups
C
Relative gene expression MYOG
4
3
2
1
0
-1
G1
G4
G7
G8
Groups
Groups
G1 (n = 4)
G4 (n = 4)
G7 (n = 5)
G8 (n = 3)
D
Relative gene expression TRIM63
7
6
5
4
3
2
1
0
-1
G1
G4
G7
G8
Groups
E
Relative gene expression TRIM55
5
4
3
2
1
0
*
G1
G4
G7
G8
Groups
F
Relative gene expression PPARG
2
1
0
-1
G1
G4
G7
G8
Groups

Figure 9

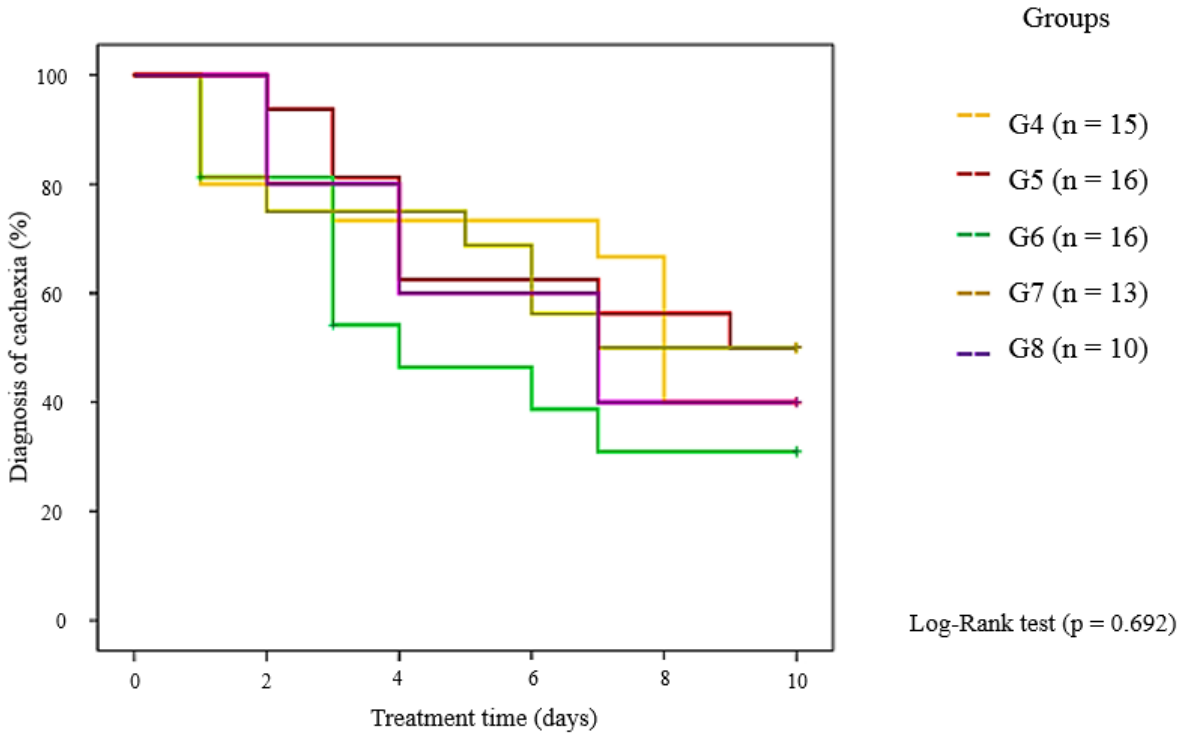

Groups
G4 (n = 15)
G5 (n = 16)
G6 (n = 16)
G7 (n = 13)
G8 (n = 10)
Diagnosis of cachexia (%)
100
80
60
40
20
0
0
2
4
6
8
10
Treatment time (days)
Log-Rank test (p = 0.692)

Figure 10

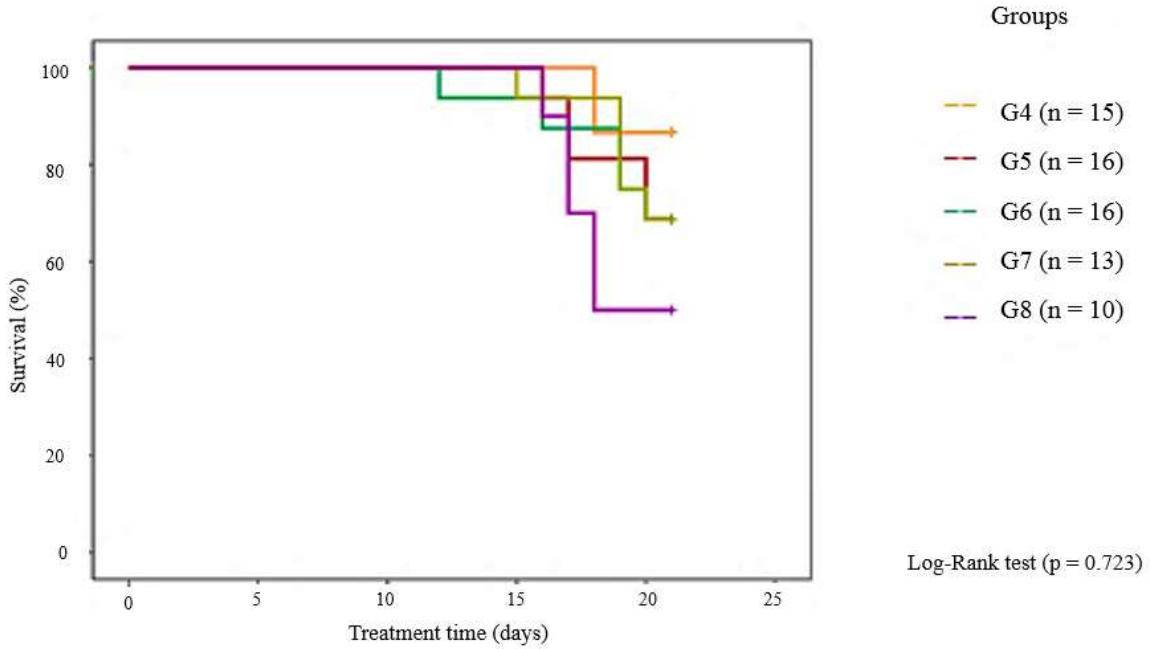

Groups
G4 (n = 15)
G5 (n = 16)
G6 (n = 16)
G7 (n = 13)
G8 (n = 10)
Survival (%)
100
80
60
40
20
0
0
5
10
15
20
25
Treatment time (days)
Log-Rank test (p = 0.723)

Table 1S. Inferential analysis of skeletal muscle strength (mean ± S.D.) and delta values (Δ) in C57BL/6 mice, sedentary controls, resistance training, physical activity practitioners and CM controls.

Groups	Skeletal Muscle Strength (g) (mean ± S.D.)			p value
	Before tumor induction	After tumor induction	Delta value (Δ)	
G1 (n=5)	76.67 ± 13.98	75.67 ± 12.61	-3.59 ± 15.30	0.627
G2 (n=5)	86.00 ± 6.58	102.00 ± 11.06	15.43 ± 15.11	0.187
G3 (n=5)	76.33 ± 10.29	88.00 ± 6.02	12.06 ± 5.59	0.008
G4 (n=15)	76.17 ± 12.02	71.17 ± 12.02	-6.56 ± 12.76	0.684

G1 = Control, sedentary. G2 = Resistance training. G3 = Physical activity. G4 = sedentary mice bearing tumor. Means of groups were compared using paired Student's T test. The delta values were analyzed analysis of variance (ANOVA) Bonferroni setting, these data did not have statistically significant differences. S.D. = standard deviation. CM = cutaneous melanoma.

Table 2S. Inferential analysis of skeletal muscle strength (mean ± S.D.) and delta (Δ) values in C57BL/6 mice, sedentary controls with CM, resistance training with CM and physical activity practitioners with CM.

.Groups	Skeletal Muscle Strength (g) (Mean ± S.D.)			p value
	Before tumor induction	After tumor induction	Delta value (Δ)	
G4 (n=15)	76.17 ± 12.33	71.17 ± 12.02	-6.06 ± 12.76	0.684
G5 (n=16)	71.06 ± 8.45	85.06 ± 24.88	19.00 ± 27.73	0.062
G6 (n=16)	66.77 ± 10.88	85.87 ± 14.97	15.00 ± 14.89	0.000
G7 (n=13)	66.33 ± 8.51	92.83 ± 11.61	27.50 ± 14.37	0.000
G8 (n=10)	60.36 ± 7.03	63.53 ± 5.03	2.50 ± 5.33	0.093

G4 = sedentary mice bearing tumor. G5 = resistance training before tumor induction. G6 = resistance training before and after tumor induction. G7 = resistance training after tumor induction. G8 = physical activity after tumor induction. CM = cutaneous melanoma. Means values for muscle strength were compared using paired Student's t test. Delta values were analyzed analysis of variance (ANOVA), with Bonferroni correction. It was noted significant differences between groups. Comparison of delta values for muscle strength between groups showed significant differences between groups G4 vs. G7 ($p = 0.005$) and G7 vs. G8 ($p = 0.019$).

Table 3S. Analysis of weight body (g), and white adipose and skeletal muscle tissues weights (g, %) in C57BL/6 mice, sedentary controls, resistance training, physical activity practitioners and CM controls.

Variables	Groups			
	G1 (n = 5)	G2 (n = 5)	G3 (n = 5)	G4 (n = 5)
BW (g)	21.00 ± 1.41	20.90 ± 1.13	21.93 ± 1.27	17.86 ± 0.61
WAT (g)	0.156 ± 0.06	0.164 ± 0.06	0.313 ± 0.14	0.120 ± 0.18
WAT (%)	0.754 ± 0.29	0.778 ± 0.31	1.466 ± 0.72	0.690 ± 1.06
SMT (g)	0.164 ± 0.06	0.294 ± 0.05	0.163 ± 0.04	0.120 ± 0.01
SMT (%)	0.774 ± 0.31	1.420 ± 0.30	0.746 ± 0.23	0.680 ± 0.10

G1 = control, sedentary. G2 = resistance training. G3 = physical activity. G4 = sedentary mice bearing tumor. BW = body weight. SMT = skeletal muscle tissue. WAT = white adipose tissue. CM = cutaneous melanoma. Means values of groups were compared using analysis of variance (ANOVA) with Bonferroni correction. S.D. = standard deviation. Significant difference for body weight variable was noted between groups G1 vs. G4 ($p = 0.022$), G2 vs. G4 ($p = 0.027$), and G3 vs. G4 ($p = 0.008$). For variable absolute SMT, significant differences were observed between G1 vs. G2 ($p = 0.004$), G2 vs. G3 ($p = 0.024$), and G2 vs. G4 ($p = 0.003$). For variable percentage SMT, significant differences were noted between G1 vs. G2 ($p = 0.017$), G2 vs. G3 ($p = 0.019$), and G2 vs. G4 ($p = 0.018$).

Table 4S. Analysis of weight body (g), and white adipose and skeletal muscle tissues weights (g, %) in C57BL/6 mice, sedentary controls with CM, resistance training with CM and physical activity practitioners with CM.

Variables	Groups				
	G4 (n = 3)	G5 (n = 3)	G6 (n = 4)	G7 (n = 4)	G8 (n = 5)
BW (g)	17.86 ± 0.61	18.33 ± 2.25	19.50 ± 1.57	24.43 ± 2.62	18.86 ± 3.45
BWTM (g)	16.00 ± 0.00	16.33 ± 1.15	17.75 ± 1.25	20.33 ± 1.15	17.00 ± 4.00
WAT (g)	0.120 ± 0.18	0.116 ± 0.02	0.055 ± 0.03	0.076 ± 0.04	0.094 ± 0.02
WAT (%)	0.690 ± 1.06	0.616 ± 0.06	0.270 ± 0.15	0.320 ± 0.21	0.498 ± 0.10
SMT (g)	0.120 ± 0.01	0.103 ± 0.02	0.112 ± 0.03	0.173 ± 0.03	0.274 ± 0.07
SMT (%)	0.680 ± 0.10	0.563 ± 0.15	0.582 ± 0.22	0.720 ± 0.186	1.472 ± 0.24
TM (g)	1.593 ± 0.48	1.922 ± 1.85	1.715 ± 0.64	4.203 ± 3.28	1.922 ± 1.85
TM (%)	8.846 ± 2.43	10.120 ± 4.56	8.725 ± 2.98	16.420 ± 11.07	9.954 ± 7.67

G4 = sedentary mice bearing tumor. G5 = resistance training before tumor induction. G6 = resistance training before and after tumor induction. G7 = resistance training after tumor induction. G8 = physical activity after tumor induction. BW = Body weight. BWTM = Body weight without tumor. SMT = skeletal muscle tissue. WAT = White adipose tissue. TM = Tumor. CM = cutaneous melanoma. Means values of groups were compared using analysis of variance (ANOVA) with Bonferroni correction. S.D. = standard deviation. Significant differences were noted between groups G4 vs. G8 ($p = 0.008$), G5 vs. G8 ($p = 0.004$), and G6 vs. G8 ($p = 0.004$) for absolute SMT values. Significant differences were noted for percentage SMT between groups ($p = 0.001$), G5 vs. G8 ($p = 0.000$), G6 vs. G8 ($p = 0.000$), and G7 vs. G8 ($p = 0.002$).

Table 5S. Analysis number and area of skeletal muscle fiber from gastrocnemius muscle in C57BL/6 mice, sedentary controls, resistance training, physical activity practitioners and CM controls.

Variables	Groups				p value					
	G1 (n = 3)	G2 (n = 3)	G3 (n = 3)	G4 (n = 3)	G1 vs. G2	G1 vs. G3	G1 vs. G4	G2 vs. G3	G2 vs. G4	G3 vs. G4
Number of cell nuclei (unity)	19.75 ± 3.99	27.8 ± 9.31	26.75 ± 16.44	22.62 ± 10.73	1.000	1.000	1.000	1.000	0.527	0.500
Number of fiber (unity)	56 ± 3.02	65 ± 9.3	60 ± 6.01	50 ± 6	1.000	1.000	1.000	1.000	1.000	1.000
Muscle fiber area (μm^2)	2892.54 ± 1206.49	3633.42 ± 1017.89	2887.19 ± 1125.20	2570.22 ± 1264.90	0.003	0.915	1.000	0.018	0.043	1.000

G1 = control, sedentary. G2 = resistance training. G3 = physical activity. G4 = sedentary mice bearing tumor. CM = cutaneous melanoma. Mean ± SD values were compared using analysis of variance (ANOVA) with Bonferroni correction.

Table 6S. Analysis number and area of adipocytes from inguinal white adipose tissue in C57BL/6 mice, sedentary controls, resisted training practitioners, physical activity practitioners and MC controls.

Variables	Groups				p value					
	G1 (n = 3)	G2 (n = 3)	G3 (n = 3)	G4 (n = 3)	G1 vs. G2	G1 vs. G3	G1 vs. G4	G2 vs. G3	G2 vs. G4	G3 vs. G4
Number of adipocytes (unity)	70.30 ± 8.00	68.10 ± 8.20	69.00 ± 7.15	65.60 ± 7.50	1.000	1.000	1.000	1.000	1.000	1.000
Adipocyte area (μm^2)	2390.38 ± 1155.79	2486.66 ± 1170.03	2396.72 ± 1187.19	2186.62 ± 1244.76	1.000	1.000	1.000	1.000	1.000	1.000

G1 = control, sedentary. G2 = resistance training. G3 = physical activity. G4 = mice bearing tumor, sedentary. CM = cutaneous melanoma. Mean ± SD values were compared using analysis of variance (ANOVA) with Bonferroni correction.

Table 7S. Analysis number and area of skeletal muscle fiber from gastrocnemius in C57BL/6 mice, sedentary controls with CM, resistance training with CM and physical activity practitioners with CM.

Variables	Groups					p value									
	G4 (n = 3)	G5 (n = 3)	G6 (n = 3)	G7 (n = 3)	G8 (n = 3)	G4 vs. G5	G4 vs. G6	G4 vs. G7	G4 vs. G8	G5 vs. G6	G5 vs. G7	G5 vs. G8	G6 vs. G7	G6 vs. G8	G7 vs. G8
Number of nuclei (unity)	16.66 ± 4.26	19.83 ± 7.65	25.5 ± 7.04	30.6 ± 8.01	28.5 ± 13.12	1.000	1.000	0.146	0.274	1.000	0.525	0.976	1.000	1.000	1.000
Number of fiber (unity)	50 ± 6	52.1 ± 5.02	59.4 ± 7.3	52.08 ± 6.1	52 ± 8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Muscle fiber area (μm^2)	2570.22 ± 1264.9	2865.54 ± 1302.26	3066.24 ± 1174.84	2465.96 ± 1101.08	2773.11 ± 1045.27	1.000	1.000	0.581	1.000	1.000	1.000	1.000	0.013	1.000	1.000

G4 = mice bearing tumor, sedentary. G5 = resistance training before tumor induction. G6 = resistance training before and after tumor induction. G7 = resistance training after tumor induction. G8 = physical activity after tumor induction. CM = cutaneous melanoma. Mean and standard deviation (S.D.) values were compared using analysis of variance (ANOVA) with Bonferroni correction.

Table 8S. Analysis number and area of adipocytes from inguinal white adipose tissue in C57BL/6 mice, sedentary controls with CM, resistance training with CM and physical activity practitioners with CM.

Variables	Groups					p value									
	G4 (n = 3)	G5 (n = 3)	G6 (n = 3)	G7 (n = 3)	G8 (n = 3)	G4 vs. G5	G4vs. G6	G4 vs. G7	G4 vs. G8	G5 vs. G6	G5 vs. G7	G5 vs. G8	G6 vs. G7	G6 vs. G8	G7 vs. G8
Number of adipocytes (unity)	65.60 ± 7.5	67.6 ± 5.66	64.00 ± 8.03	72.44 ± 17.09	68.38 ± 9	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Adipocyte area (μm^2)	2186.62 ± 1244.76	2639.59 ± 1293.76	2170.08 ± 1014.54	1937.27 ± 1178.57	2565.52 ± 1163.13	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

G4 = mice bearing tumor, sedentary. G5 = resistance training before tumor induction. G6 = resistance training before and after tumor induction. G7 = resistance training after tumor induction. G8 = physical activity after tumor induction. CM = cutaneous melanoma. Mean and standard deviation (S.D.) values were compared using analysis of variance (ANOVA) Bonferroni setting.

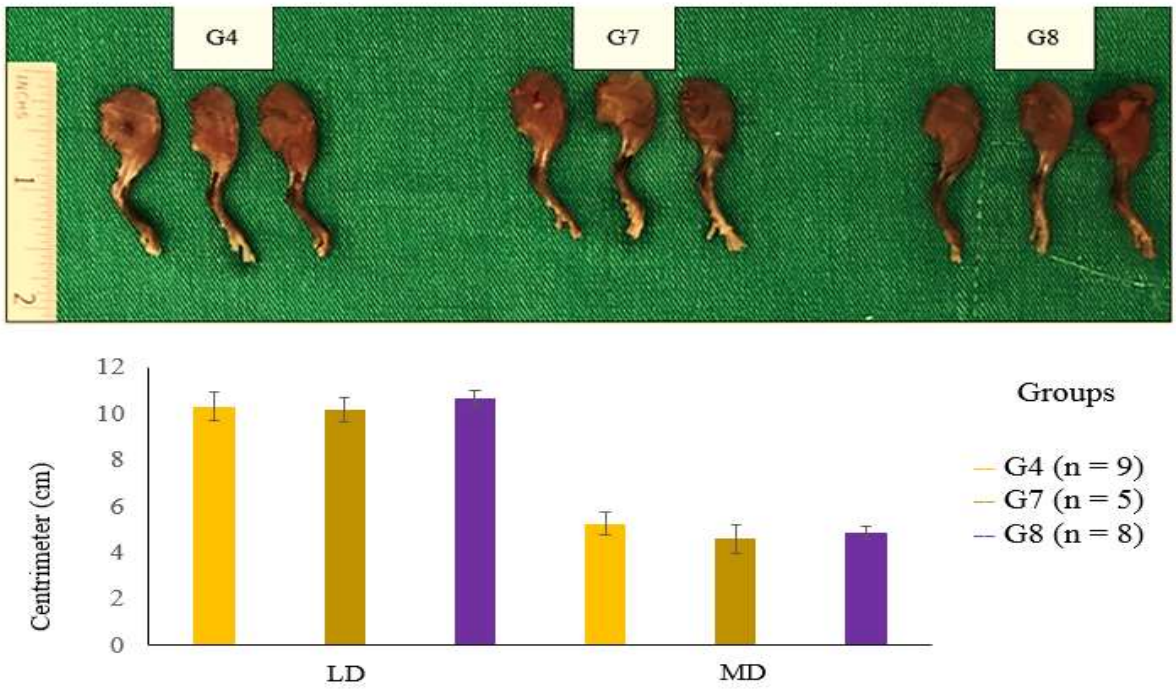
Table 9S - Analysis of right hindlimb volume (mm^3) obtained with ultrasound device in control and C57BL/6 mice bearing tumor submitted to resistance training, physical activity, and sedentary.

Groups	Muscle Volume			p value	p value - delta value (Δ)		
	Initial (10 days after tumor induction)	Final (19 days after tumor induction)	Delta value (Δ)		G4 vs G7	G4 vs G8	G7 vs G8
G4 (n = 9)	0.085 ± 0.00	0.072 ± 0.00	-0.012 ± 0.00	0.001			
G7 (n = 5)	0.072 ± 0.02	0.081 ± 0.01	0.008 ± 0.02	0.277	0.024	0.957	0.026
G8 (n = 8)	0.086 ± 0.04	0.070 ± 0.02	-0.016 ± 0.01	0.000			

G4 = mice bearing tumor, sedentary. G7 = resistance training after tumor induction. G8 = physical activity after tumor induction. Means values of groups were compared using paired Student's T test. The delta values were analyzed using analysis of variance (ANOVA), with Bonferroni correction. S.D. = standard deviation.

Figure S1. Analysis of limb diameter (cm) of C57BL/6 mice of groups G4, G7 and G8 on right hind limb. Means of groups were compared using analysis of variance (ANOVA) Bonferroni setting, these data did not have statistically significant differences. MD = Minor diameter. LD = Larger diameter. Mean and standard deviation values (D.P.) were compared using ANOVA of Bonferroni configuration, the findings did not present a significant difference.

Figure S1



4 CONSIDERAÇÕES FINAIS

A investigação cientifica realizada nesse tese, mostrou resultados importantes sobre o uso do dispositivo de escada vertical com estimulação por choque elétrico para similaridade do exercício resistido, uma vez que os achados mostram que o protótipo apresentou semelhanças nas adaptações agudas e crônicas esperadas para o treinamento resistido.

Com relação aos experimentos realizados com camundongos C57BL/6 com caquexia associada ao melanoma cutâneo, os resultados mostram que não ocorreu contribuição significativa do treinamento resistido e da atividade física na quadro caquético e na sobrevida global. Mas ocorreu contribuições na força muscular, composição corporal e na histomorfometria. Porém os achados da expressão gênica não mostram diferença significativa, pois acreditamos que o controle de carga deva ser modificado para melhor rendimento dessas variáveis.

Portanto, novos estudos devem ser encorajadas, pois propostas de outras investigações fisiológicas e moleculares poderão elucidar as lacunas aqui deixadas.

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ANEXO A - Parecer consubstanciado da comissão de ética em experimentação e bem-estar animal.

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
COMISSÃO DE ÉTICA EM EXPERIMENTAÇÃO E BEM-ESTAR ANIMAL **Unimontes**

PARECER CONSUBSTANCIADO

Montes Claros, 20 de fevereiro de 2017.

Processo N.º 131

Título do Projeto: **EFEITOS DO EXERCÍCIO FÍSICO, DO RESVERATROL NO SISTEMA UBIQUINA-PROTEASSOMA, NO CRESCIMENTO TUMORAL, NA OCORRÊNCIA DE METÁSTASE E NA CAQUEXIA ASSOCIADA AO MODELO SINGÊNICO DE MELANOMA CUTÂNEO EM CAMUNDONGOS C57Bl/6.**

Orientador: **Prof. Dr. Alfredo Maurício Batista de Paula**

Histórico
O melanoma humano representa um tipo de câncer que ocorre a partir de alterações moleculares genéticas e epigenética em melanócitos localizados nos revestimentos mucoso e cutâneo e em órgãos internos. O melanoma cutâneo (MC) apresenta altas taxas de prevalência e de incidência em várias populações em todo o mundo sendo, portanto, um importante problema de saúde pública mundial. Frequentemente, o MC apresenta altas taxas de morbidade e mortalidade devido à sua usual precocidade de disseminação metastática. Uma parcela importante dos pacientes com MC manifesta uma síndrome paraneoplásica conhecida como caquexia, caracterizada como uma progressiva consumação física decorrente principalmente de alterações do metabolismo proteico em fibras musculares esqueléticas. A caquexia promove um impacto significativamente negativo sobre a resposta terapêutica antineoplásica, a qualidade de vida e o tempo de sobrevida de indivíduos com câncer. Embora há muito reconhecida clinicamente, a caquexia associada ao câncer não responde às terapias atuais devido à complexidade dos distúrbios moleculares associadas a essa condição clínica.

Mérito
Avaliar os efeitos do exercício físico, do resveratrol no sistema ubiquinaproteassoma, no crescimento tumoral, na ocorrência de metástase e na caquexia associada ao modelo singênico de melanoma cutâneo em camundongos C57Bl/6.
Baseada nas Informações contidas no projeto e no Protocolo para uso de animais em Pesquisa o Projeto tem Mérito.

Parecer
A Comissão de Ética em Experimentação e Bem-Estar Animal da Unimontes analisou o processo **131** e entende que o mesmo está dentro das normas da Comissão Sendo assim, somos pela **APROVAÇÃO** do projeto de pesquisa.

Prof. Orlando Raphael Lopasso Júnior
Presidente da Comissão de Ética em Experimentação
e Bem-Estar Animal da UNIMONTES

ANEXO B - Peticionamento eletrônico (modelo de utilidade)

INPI INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

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Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 20 2018 015810 0

Dados do Depositante (71)

Depositante 1 de 1

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Dados do Pedido

Natureza Patente: 20 - Modelo de Utilidade (MU)

Título da Invenção ou Modelo de Utilidade (54): ESCADA COM DISPOSIÇÕES INTRODUZIDAS PARA TREINAMENTO NEUROMUSCULAR RESISTIDO DE ROEDORES

Resumo: A presente patente de modelo de utilidade trata de nova forma de escada (1,1 x 0,18 m; 43 degraus, de 2 cm cada; inclinação de 80º), constituída, na parte superior, no topo da escada, de câmara de descanso e câmara de alimentação, e, na parte inferior (base ou área de saída), disposições introduzidas, contendo estimulador elétrico, painel de controle de estimulação elétrica, câmara de estimulação e contenções laterais ao longo dos degraus. É direcionada a empresas que investem no desenvolvimento e comercialização de produtos voltados à pesquisa experimental animal, educadores e pesquisadores de Instituições de Ciência, Tecnologia e Inovação (ICT) cujos projetos de ensino e pesquisa pretendem avaliar efeitos que ocorrem na musculatura esquelética dos camundongos e ratos, a partir dos treinamentos neuromusculares resistidos.

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Email: marianarochaalves13@gmail.com

Documentos anexados

Tipo Anexo	Nome
Comprovante de pagamento de GRU 200	Comprovante de Pagamento da GRU.pdf
Desenho	Desenhos.pdf
Resumo	Resumo.pdf
Declaração de período de graça	EFEITO DO TREINAMENTO RESISTIDO NA FORÇA MUSCULAR DE CAMUNDONGOS C57BL_6COM CAQUEXIA ASSOCIADA AO MELANOMA CUTÂNEO EXPERIMENTAL SINGÊNICO.pdf
Declaração de período de graça	EFEITO DO TREINAMENTO RESISTIDO NO VOLUME TUMORAL DO MELANOMA CUTÂNEO EXPERIMENTAL SINGÊNICO EM CAMUNDONGOS C57BL_6.pdf
Portaria	Portaria 035 Reitor 2012 Delega Competência Pró-Reitor INPI.pdf
Portaria	portaria_reitor023_Prof Virgilio.pdf
Relatório Descritivo	Relatório Descritivo.pdf

Acesso ao Patrimônio Genético

☑ Declaração Negativa de Acesso - Declaro que o objeto do presente pedido de patente de invenção não foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético Brasileiro, o acesso foi realizado antes de 30 de junho de 2000, ou não se aplica.

Declaração de Divulgação Anterior Não Prejudicial

☑ Artigo 12 da LPI - Período de Graça.

Declaração de veracidade

☑ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.

Governo do Estado de Minas Gerais

S I A F I - MG

Ordem de Pagamento Bancária

Matrícula: X003969
Data Impressão: 06/02/2018
Hora Impressão: 16:15:11
Pág.: 1 / 2

Nº do Documento: 1375 **Nº Documento Global:** 1187 **Data do Registro:** 01/02/2018 **Hora:** 11.30.04
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Ano Exercício: 2018

Credor da OP: 42521088/0001-37 - INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL
Credor do Empenho: 42521088/0001-37 - INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

DADOS DO PAGAMENTO1

Data de Pagamento: 02/02/2018 **Tipo de Pagamento:** TITULO OUTRO BANCO
Conta Débito: Banco: 341 Agência: 3380- Conta: 60000-1 CONTA UNICA ESTADUAL
Conta Crédito: Banco: 1 Agência: 0 Conta: 0
Valor Bruto: 70,00 **Valor Desc.:** 0,00 **Valor IRRF:** 0,00
Valor Multa: 0,00 **Valor Taxa:** 0,00 **Valor Patr.:** 0,00 **Valor Pgto.:** 70,00

DADOS ADICIONAIS

Código de Barras: 00190000090294091618801047994171174500000007000
Origem: EMPENHO
Processo de Sentença Judicial: 0 **Parcelas:** 0
Finalidade de Pagamento:
Operador do Registro: X003969 - LUIZ PEREIRA DA SILVA
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HISTÓRICO DE REFERÊNCIA

REF. DOCUMENTO 29409161801047994 - TAXA DO PEDIDO DE NOVA PATENTE"ESCADA COM ESTIMULADOR ELETRICO PARA TREINAMENTO RESISTIDO DE CAMUNDONGOS E RATOS".

DETALHAMENTO DA ORDEM DE PAGAMENTO BANCÁRIA

Nº	Tipo	Número	Ano Origem	GMI FP	Elem./Item	Evento	LCP	IPC	IRRF	Valor Pago
1	EMPENHO	3654	2017	3900 101	47/01	701002	1070	22	N	70,00

ASSINATURA DIGITAL DO ORDENADOR DA DESPESA

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Autenticação Eletrônica: 25C370A393DF39A1BFE77A53D90107B0C104CD269919B93CCF491FD900723A6C
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Tipo de Pagamento: TITULO OUTRO BANCO
Unidade de Registro:

ANEXO C – Carta de aceite da revista "Journal of Human Sport and Exercise"

JHSE
JOURNAL OF HUMAN SPORT AND EXERCISE
University of Alicante
Official International Network Journal on Sport and Health Sciences
www.jhse.ua.es ISSN 1988-5202 DOI 10.14198/jhse

José Antonio Pérez Turpin, as Editor-in-Chief of Journal of Human Sport and Exercise,

CERTIFIES

that Vinicius Dias Rodrigues, Daniel de Moraes Pimentel, Andréia de Souza Brito, Magda Mendes Vieira, Amanda Rodrigues Santos, Amanda Souto Machado, Lorrane Katherine Martins Pereira, Fernanda Santos Soares, Emisael Stênio Batista Gomes, Mariana Rocha Alves, Ludmilla Regina de Souza, Ricardo Cardoso Cassilhas, Renato Sobral Monteiro Júnior and Alfredo Maurício Batista De-Paula are the authors of article entitled: "*Methodological validation of a vertical ladder with low intensity shock stimulus for resistance training in C57BL/6 mice: Effects on muscle mass and strength, body composition, and lactate plasma levels*", which has been reviewed and accepted by the Editorial Board and will be published in the **Journal of Human Sport and Exercise**, whose ISSN is 1988-5202 and is included (among others) in the following databases and assessment systems:

ESCI – WoS
Scopus
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RESH
Latindex
EBSCO - SPORTDiscus
Redalyc
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Google Scholar
ISOC. Database of CINDOC – CSIC
Directory of Open Access scholarly Resources (ROAD)
Olympic World Library (IOC)

Alicante (Spain) at November 9th, 2018

José Antonio Pérez Turpin - *Editor-in-Chief*
Journal of Human Sport and Exercise