

**UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE
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Denielli da Silva Gonçalves Bós

**EXERCÍCIO AERÓBICO DE BAIXA INTENSIDADE MELHORA A FUNÇÃO
HEMODINÂMICA E A MECÂNICA RESPIRATÓRIA EM RATOS COM
HIPERTENSÃO ARTERIAL PULMONAR**

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“Determinação, coragem e autoconfiança são fatores decisivos para o sucesso. Não importa quais sejam os obstáculos e as dificuldades. Se estamos possuídos de uma inabalável determinação, conseguiremos superá-los. Independentemente das circunstâncias, devemos ser sempre humildes, recatados e despidos de orgulho”.

Dalai Lama

RESUMO

O exercício aeróbico tem demonstrado benefícios em pacientes com Hipertensão Arterial Pulmonar (HAP), melhorando a dispneia e o aumento da força muscular ventilatória. Portanto, o objetivo do estudo foi avaliar o efeito de diferentes intensidades de exercício aeróbico sobre a mecânica respiratória e a função hemodinâmica em ratos com HAP. Ratos Wistar foram induzidos à HAP através de dose única de monocrotalina (50mg/kg), divididos em três grupos: HAP-sedentários (HAP-S; n=5); HAP-treinamento intensidade baixa (HAP-B; 40% da velocidade máxima teste de esforço; n=6); HAP-treinamento intensidade moderada (HAP-M; 60% da velocidade máxima teste de esforço; n=8). O protocolo de exercício aeróbico consistiu em caminhada em esteira por três semanas (5x/semana/50min). Após três semanas, foram traqueostomizados, para a análise da mecânica respiratória (FlexiVent) e cateterizados para avaliação hemodinâmica. Os dados foram comparados por ANOVA de uma via seguida pelo teste Student-Newman-Keuls ($P<0,05$). Os grupos submetidos ao treinamento aeróbico tiveram uma pressão ventricular sistólica direita significativamente ($P<0,05$) menor quando comparados aos animais sedentários (HAP-S= $40\pm 9,6$; PAH-L= $25\pm 2,6$; PAH-M= $29\pm 5,1$; mmHg). A elastância estática foi menor no grupo HAP-B quando comparado ao HAP-M (HAP-B= $0,71\pm 0,05$ vs. HAP-M= $0,98\pm 0,16$; cmH₂O/mL) e a elastância dinâmica foi menor no HAP-B quando comparado ao HAP-S (HAP-B= $1,47\pm 0,14$ vs. HAP-S= $1,91\pm 0,31$; cmH₂O/mL). Complacência estática foi maior no grupo HAP-B (HAP-B= $1,40\pm 0,10$ vs. HAP-S= $1,17\pm 0,14$; mL/cmH₂O) e menor no HAP-M (HAP-M= $1,05\pm 0,16$ vs. HAP-B= $1,47\pm 0,14$; mL/cmH₂O), complacência dinâmica foi maior no grupo HAP-B (HAP-B= $0,68\pm 0,06$ vs. HAP-S= $0,53\pm 0,09$; mL/cmH₂O) em relação ao HAP-M (HAP-B= $0,68\pm 0,06$ vs. HAP-M= $0,57\pm 0,05$; mL/cmH₂O). O resultado mais relevante desse estudo foi que o treinamento aeróbico diminuiu significativamente a pressão ventricular direita nos ratos com HAP, no entanto, apenas o exercício de baixa intensidade melhorou a mecânica respiratória. Coletivamente, esses resultados indicam que o exercício de baixa intensidade é eficaz para reduzir a pressão no ventrículo direito e melhorar a condição cardiorrespiratória na HAP.

Palavras-chave: Hipertensão Pulmonar. Monocrotalina. Exercício. Mecânica respiratória.

ABSTRACT

Aerobic exercise has been beneficial in patients with Pulmonary Arterial Hypertension (PAH), improving dyspnea and increasing ventilatory muscle strength. On the other hand, it is not very well known which intensity of exercise has better effects on respiratory mechanics and hemodynamic function and less risk to PAH patients. PAH was induced in Wistar rats by a single dose of monocrotaline (50mg/kg; s.c.) in three different groups: PAH-sedentary rats (PAH-S; n=5) and exercised rats in a treadmill for three weeks (5x/week/50min) at low intensity (PAH-L, 40% of maximum speed in incremental test; n=6) and moderate intensity of exercise-training (PAH-M, 60% of the maximum speed in incremental test; n=8). After 3 weeks, rats were tracheostomized to study the respiratory mechanics (FlexiVent) and catheterized for hemodynamic evaluation. Data were compared by one-way ANOVA test followed by Student-Newman-Keuls test ($P<0.05$). Exercise in both intensities significantly decreased ($P<0.05$) right ventricular systolic pressure elevation induced by monocrotaline (RVSP) (PAH-S= 40 ± 9.6 ; PAH-L= 25 ± 2.6 ; PAH-M= 29 ± 5.1 ; mmHg) compared to PAH-S. The quasi-static elastance was significantly lower in PAH-L *versus* PAH-M group (0.71 ± 0.05 vs. 0.98 ± 0.16 ; cmH₂O/mL), whereas the respiratory elastance was lower in the PAH-L *versus* PAH-S group (1.47 ± 0.14 vs. 1.91 ± 0.31 ; cmH₂O/mL). Quasi-static compliance was significantly higher in PAH-L *versus* PAH-S (1.40 ± 0.10 vs. 1.17 ± 0.14 ; mL/cmH₂O) and lower in PAH-M *versus* PAH-L (1.05 ± 0.16 vs. 1.47 ± 0.14 ; mL/cmH₂O). The respiratory system compliance was higher in PAH-L either when compared to the sedentary and moderate groups (PAH-L= 0.68 ± 0.06 vs. PAH-S= 0.53 ± 0.09 , and vs. PAH-M= 0.57 ± 0.05 ; mL/cmH₂O, $P<0.05$). Collectively, these results indicate that low intensity of an aerobic exercise is effective to decrease the RVSP, which results in a better cardiorespiratory condition during PAH.

Keywords: Pulmonary hypertension. Monocrotaline. Exercise. Respiratory mechanics.

LISTA DE ABREVIATURAS

CP	Complacência Pulmonar
DPOC	Doença pulmonar obstrutiva crônica
HAP	Hipertensão arterial pulmonar
IC	Insuficiência cardíaca
MCT	Monocrotalina
OMS	Organização Mundial da Saúde
PDFVD	Pressão diastólica final no ventrículo direito
VD	Ventrículo direito
VEGF	Fator de crescimento vascular endotelial

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1 INTRODUÇÃO

A Hipertensão arterial pulmonar (HAP) ocorre como um fenômeno isolado ou associado a várias alterações cardiopulmonares, sendo consequência de uma série de patologias, como a insuficiência cardíaca (IC), a doença pulmonar obstrutiva crônica (DPOC), a fibrose cística, a fibrose pulmonar idiopática, entre outras (1). É caracterizada pelo aumento progressivo da resistência dos vasos pulmonares, levando à falência do ventrículo direito (VD) e morte súbita, tendo uma elevada mortalidade após o diagnóstico (2).

O seu diagnóstico geralmente ocorre em fases avançadas das doenças que são associadas à HAP, responsáveis pela maioria dos sinais e sintomas, tais como a intolerância ao exercício, a dispneia e a fadiga, decorrentes do baixo débito cardíaco de caráter progressivo e da ineficiência na oferta de oxigênio (3-5). Em conjunto, esses achados levam o paciente a limitar as suas atividades diárias com consequente redução da qualidade de vida (6).

Dessa forma, a prática de exercício físico supervisionado pode ser benéfica nesses indivíduos, possivelmente por melhorar a demanda e a utilização de oxigênio pelos músculos em atividade, além de reduzir a dispneia e a fadiga, gerando melhora do desempenho na realização de suas atividades diárias.

Estudos com ratos, utilizando a monocrotalina (MCT) para induzir a HAP, têm contribuído muito para maior compreensão dessa doença (7, 8). A MCT é um alcaloide, extraído da planta *Crotalaria* sp., que produz dano endotelial, edema e remodelamento pulmonar, além de hipertrofia do VD (9, 10), resultando na HAP.

Uma das principais vantagens da utilização do modelo com MCT, é que os resultados apresentados por animais tratados com esta droga mimetizam várias alterações fisiopatológicas apresentadas por humanos portadores de HAP primária. Deste modo, é considerado um dos modelos apropriados para estudar em maiores detalhes a fisiopatologia do desenvolvimento da HAP, com a finalidade de identificar novas estratégias terapêuticas em humanos (7, 11).

Considerando a elevada mortalidade na HAP, e que o tratamento para essa doença permanece elusivo, o desenvolvimento de tratamentos alternativos para controlá-la representa um grande desafio. Um estudo realizado em pacientes com HAP, classes III e IV, em um centro de referência, verificou que o uso isolado de

estratégias terapêuticas farmacológicas avançadas obteve uma taxa de sobrevida de 93% e 83,1% no primeiro e segundo ano, respectivamente (12)

No entanto, estudos que avaliaram o efeito do uso de medicação associada ao exercício físico demonstraram melhores resultados do que o uso da medicação isoladamente. Esse fato fica claramente demonstrado no estudo de Grünig e colaboradores (2011) que observaram, em pacientes que realizaram treinamento físico associado ao tratamento medicamentoso, uma taxa de sobrevida de 100% no primeiro ano e de 95% no segundo ano (13). Dessa forma, fica claro o impacto do exercício na sobrevida dos pacientes, a qual no segundo ano aumentou em 12% nos pacientes com HAP que praticaram exercício físico.

Acreditamos que o exercício físico de baixa intensidade possa promover efeitos benéficos nesse modelo de HAP, melhorando a função respiratória e o sistema cardiovascular. No entanto, não está claro na literatura qual a melhor intensidade de treinamento aeróbico que poderá contribuir para aumentar a taxa de sobrevida, com baixo risco, aos pacientes com HAP.

De fato, evidências atuais têm demonstrado que o exercício pode ser benéfico ao paciente com HAP, pois melhora a qualidade de vida e o aporte de oxigênio aos tecidos (13-16). Sendo assim, o objetivo desse estudo é avaliar, na HAP induzida por MCT, os efeitos de duas intensidades de exercício físico aeróbico, o de baixa e o de moderada intensidade, sobre a mecânica respiratória e a hemodinâmica cardíaca. Ao longo dessa revisão da literatura, serão abordadas a definição, fisiopatologia, sobrevida e mortalidade em consequência da HAP. Da mesma forma abordaremos o modelo de indução de HAP em animais, mecânica respiratória, atuação do exercício sobre a circulação pulmonar, efeito de diferentes protocolos de exercício e o impacto do exercício sobre a mecânica respiratória na HAP.

2 HIPERTENSÃO ARTERIAL PULMONAR

A HAP é uma síndrome resultante da circulação restrita na artéria pulmonar, o que leva ao aumento da pressão nessa artéria, sendo caracterizada pela presença de pressão média maior do que 25 mmHg em repouso e maior do que 30 mmHg com o exercício (17-19). O seu diagnóstico geralmente ocorre na fase avançada da doença com sintomas como a intolerância ao exercício, dispneia e fadiga decorrentes do baixo débito cardíaco, que tem caráter progressivo, levando à ineficiência na oferta de oxigênio aos tecidos (3, 5, 20). Em conjunto, esses achados levam o paciente a limitar as suas atividades de vida diária com consequente redução da qualidade de vida (6).

É caracterizada pelo aumento progressivo da resistência dos vasos pulmonares, levando à hipertrofia patológica do VD, falência do VD e em último estágio à morte súbita (1, 20).

A causa predominante de aumento da pressão é a diminuição da secção transversal do lúmen da artéria pulmonar, devido ao remodelamento vascular. Além disso, a vasoconstrição desempenha um papel significativo em aproximadamente 20% dos pacientes (21, 22).

De fato, na HAP ocorre uma disfunção do VD, através do remodelamento patológico, gerando hipertrofia e aumento da pressão no ventrículo. Essa disfunção já foi descrita em vários estudos, como estando relacionada com o prognóstico e gravidade da HAP (23-25). van Wolferen e colaboradores (2007) demonstraram que um baixo volume sistólico no VD e, consequentemente, uma baixa pressão sistólica, são fatores preditores para sobrevida dos pacientes com HAP (25). Esse fato possui uma relevância clínica, pois fornece um parâmetro direto de predição de uma insuficiência do VD, que permite uma avaliação dos efeitos do tratamento, seja ele farmacológico ou não.

Dessa forma avaliar a função ventricular direita é de real importância, uma vez que a redução da pressão demonstra um melhor prognóstico para esses pacientes. Além disso, considerando que a prática de exercício físico supervisionado pode ser benéfica nesses indivíduos, acreditamos que o exercício pode representar uma alternativa bastante eficaz no sentido de melhorar a qualidade de vida, função do VD e a sobrevida dos pacientes com HAP.

3 CLASSIFICAÇÃO DA HIPERTENSÃO ARTERIAL PULMONAR SEGUNDO A OMS E SUA PREVALÊNCIA

A HAP é uma doença considerada rara, com uma prevalência de 15 casos em um milhão (19). A HAP pode ter diversas causas e origens, ela também pode não ter causa conhecida, sendo assim caracterizada como HAP idiopática. Pode ser também de origem familiar, genética, associada à doença cardíaca congênita, doenças no tecido conectivo, ou por ingestão de drogas e toxinas que levam à doença (17).

A HAP idiopática possui uma prevalência de seis casos por milhão (26). Um estudo longitudinal recente, no Reino Unido, demonstrou uma incidência da HAP idiopática e genética de 6,6 casos por milhão em 2009, sendo 92,9% dos casos HAP idiopática e 5,4% dos casos genéticos, com uma taxa de sobrevida no primeiro, segundo e terceiro ano após diagnóstico, de 92,9%, 84% e 73,3%, respectivamente (27). No Brasil, um estudo realizado em dois centros de referência em São Paulo observou que aproximadamente 62% dos indivíduos se encontravam em classe funcional III e IV, sendo 50% diagnosticados com HAP idiopática, 30% associada à esquistossomose, e 10% associada a doenças do tecido conectivo (28).

Todas as etiologias da HAP estão classificadas em cinco grupos, de acordo com a Organização Mundial de Saúde (OMS), onde, no Grupo I, a HAP ocorre por um conjunto de síndromes que refletem na doença da artéria pulmonar, no entanto nesse grupo incluem-se síndromes distintas como a HAP idiopática, associada à esquistossomose, e por doença cardíaca congênita. No Grupo II, a HAP é secundária à elevação da pressão no átrio esquerdo relacionada com doença cardíaca esquerda. No grupo III, resulta de doenças pulmonares e/ou hipoxemia crônica. No grupo IV, doença embólica e/ou tromboembolismo crônico. E no grupo V, diversas outras etiologias de HAP, como a sarcoidose e obstrução da artéria pulmonar (1).

A HAP do Grupo I, segundo a OMS, ocorre de forma rara, sendo classificada como familiar em 10% dos casos e esporádica em 90%, e de uma forma mais comum associada ao vírus HIV, doenças do tecido conectivo, doença cardíaca congênita (29). Apesar de recentes avanços nas terapias e dos sintomas melhorados dos pacientes, a HAP ainda tem uma sobrevida de apenas 60% em

cinco anos (30). A incidência estimada da HAP é de 2,4 a 7,6 casos por milhão ao ano (26, 31). A incidência do grupo II é indefinida, embora esse grupo seja, certamente, mais numeroso. A prevalência do grupo III é estimada em 1-3/10000 (32). Já a do grupo IV, após um ano com êmbolo pulmonar agudo, é cerca de 3%, com a maioria dos tromboembolos evoluindo para a HAP crônica (33).

De maneira geral, a HAP possui um mau prognóstico, com uma mortalidade no primeiro ano após o diagnóstico de aproximadamente 15%, mesmo com tratamento medicamentoso moderno (34). Os preditores de prognóstico são: a capacidade ao exercício, medida através da distância percorrida no teste de caminhada de seis minutos ou através do teste cardiopulmonar; elevada pressão atrial direita, que demonstra o grau de disfunção ventricular direita; evidência de falência do VD e baixo débito cardíaco.

Tendo em vista a alta taxa de mortalidade após o diagnóstico de HAP, e que um dos principais preditores para o pior prognóstico é a incapacidade ao exercício, uma das alternativas promissoras para amenizar esse prognóstico reservado dos indivíduos com HAP é a implementação da prática de exercício físico aeróbico como coadjuvante da terapia medicamentosa (17, 35), para melhorar a capacidade aeróbica e consequentemente aumentar a tolerância ao exercício, e indiretamente melhorar o prognóstico.

4 INDUÇÃO DE HIPERTENSÃO ARTERIAL PULMONAR POR MONOCROTALINA

Modelos animais são importantes, pois permitem o estudo de alguns dos diversos grupos de HAP. Um dos modelos, bem conhecido e utilizado, é a indução da HAP através da MCT. A MCT é um alcaloide extraído da semente da planta *Crotalaria* sp., primeiramente descrito por Bras e colaboradores em 1954. Esses pesquisadores, estudando jamaicanos que usavam ervas medicinais contendo a planta *Crotalaria* sp., demonstraram a implicação do uso desse alcaloide na doença vaso-oclusiva pulmonar (36, 37).

A MCT sofre oxidação no fígado para formar o pirrol da monocrotalina. Através de um mecanismo desconhecido, induz rapidamente doença vascular pulmonar grave que, durante um período de uma a duas semanas, é seguido pelo remodelamento vascular pulmonar e elevação da pressão na artéria pulmonar, induzindo dano endotelial, edema e hipertrofia do VD (9, 10, 37).

Uma única dose de MCT em ratos, variando entre 60 e 100 mg/kg por via subcutânea ou intraperitoneal resulta em HAP em três semanas e leva à morte dentro de seis a oito semanas (38). De acordo com a literatura, uma dose de 60 mg/kg leva à hipertrofia ventricular direita em torno de 8 semanas, em 6 semanas já se observa fibrose ventricular direita, e de 4 a 8 semanas já é possível observar redução do peso corporal e aumento da massa pulmonar. A progressão da doença é tempo-dependente e varia de forma significativa de acordo com a sensibilidade de cada animal (39).

Está bem descrito que a utilização de 50 mg/kg gera HAP em modelos animais, sem no entanto, apresentar alta mortalidade ainda nas primeiras semanas. É capaz ainda de elevar a pressão sistólica do VD, aumentar a espessura da parede dos vasos pulmonares, gerar fibrose intersticial e menor mortalidade decorrente da progressão da doença nas primeiras semanas (40, 41), razão pela qual nesse estudo optamos por essa dosagem.

As doses de MCT devem respeitar a sensibilidade de cada cepa de rato, de maneira que é possível encontrar na literatura diversas doses desse fármaco provocando HAP. Além disso, segundo a literatura, a HAP pode ser caracterizada como estável ou progressiva. Dependendo da dose de MCT administrada e do tempo de progressão da doença (42) é possível estudar tanto o estágio estável como o progressivo da doença.

De fato, estudos com ratos têm contribuído muito para uma maior compreensão dessa doença (7, 8). Uma das principais vantagens da utilização do modelo de HAP com MCT é que os resultados apresentados por animais tratados com essa droga mimetizam várias alterações fisiopatológicas apresentadas por humanos portadores de HAP primária. Desse modo, é considerado um dos modelos apropriados para estudar em maiores detalhes a fisiopatologia da HAP (37) na tentativa de identificar novas estratégias terapêuticas para seres humanos, sejam elas farmacológicas ou não.

5 CIRCULAÇÃO PULMONAR DURANTE O EXERCÍCIO

Normalmente, a circulação pulmonar é caracterizada por baixas pressões e alto fluxo. A baixa pressão evita que o fluido saia dos vasos pulmonares para o espaço intersticial e permite que o VD bombeie sangue eficientemente com um baixo gasto de energia.

Durante o repouso, o débito cardíaco varia de 4 a 6 litros/min, já durante o exercício físico, ocorre um aumento da demanda na oferta de oxigênio para os tecidos, que é suprida através do aumento do débito cardíaco, em torno de 3 a 4 vezes dos volumes de repouso. Para suportar esse aumento de fluxo sanguíneo, o leito vascular pulmonar possui a capacidade de se dilatar de forma significativa. Essa capacidade se deve a baixa resistência e a alta complacência desse leito, garantindo uma adaptação profundamente necessária às variações hemodinâmicas que as atividades diárias impõem a circulação cardiopulmonar (43). Dessa forma, qualquer alteração dessa capacidade adaptativa pode ter consequências sérias na qualidade de vida dos pacientes com HAP.

O aumento do débito cardíaco durante o exercício é necessário para suprir as crescentes demandas de transporte dos gases para os músculos em atividade. O exercício pode ser considerado um estresse considerável sobre a circulação pulmonar que deve manter a função do VD compensada e os pulmões secos. A pressão na artéria pulmonar aumenta durante o exercício e, conseqüentemente a pressão no VD deve se adaptar para garantir a adequada oxigenação dos tecidos (44). Por essa razão, por muito tempo a prática de exercício físico foi considerada muito arriscada nos indivíduos com HAP, que já possuem uma pressão na artéria pulmonar elevada.

O fluxo através do sistema cardiopulmonar é o resultado das diferenças na pressão ou gradientes entre as duas extremidades do sistema, a artéria pulmonar (20 mmHg) e o átrio esquerdo (12 mmHg). Para o VD existem várias possíveis formas de estimar a pré-carga, entre as quais a pressão diastólica final (PDFVD) ou o volume diastólico final representam as medidas mais importantes. A PDFVD pode ser avaliada clinicamente através da medição direta da pressão no VD usando um cateter na artéria pulmonar (45).

Uma das primeiras descrições da resposta do leito vascular pulmonar ao exercício foi feita por Hickam e Cargill (1948), que mostraram que durante um exercício de baixa intensidade, quando o débito cardíaco está aumentado de uma forma limitada, há pequenos aumentos na pressão média da artéria pulmonar (46).

Em indivíduos saudáveis, o débito cardíaco pode aumentar mais de quatro vezes com o exercício, enquanto a pressão da artéria pulmonar normalmente aumenta cerca de duas vezes (47).

Em um estudo com mais de 600 atletas treinados para resistência e força, o limite superior da pressão sistólica da artéria pulmonar foi de 40 mmHg. Esse resultado foi considerado uma resposta fisiológica do exercício físico intenso em longo prazo (48). Em estudo posterior, foram avaliados 124 voluntários saudáveis, sendo 62 mulheres e 62 homens jovens, em repouso e durante exercício. Nesse estudo foi observado que durante o pico de exercício houve um aumento de cerca de quatro vezes no débito cardíaco, enquanto a pressão média da artéria pulmonar dobrou (49). Esse achado claramente demonstra que, sob condições fisiológicas, a vasculatura pulmonar tem a capacidade de “amenizar” grandes variações de volume nessa circulação, um indicativo da complacência dessa vasculatura.

Outro estudo avaliou a pressão na artéria pulmonar através de ecocardiografia, em atletas condicionados e em indivíduos com doença pulmonar obstrutiva moderada, em repouso e durante ciclo de exercício. A pressão sistólica da artéria pulmonar foi 10 mmHg superior em indivíduos com doença pulmonar em comparação ao grupo de atletas em repouso (50), o que confirma a elevada complacência do sistema em indivíduos saudáveis.

Pacientes com HAP têm um padrão semelhante de resposta ao exercício como os pacientes com IC e HAP secundária à doença cardíaca do lado esquerdo. Com o aumento da resistência vascular pulmonar e a progressão da doença, ocorre uma diminuição no débito cardíaco e na distribuição de oxigênio para o músculo esquelético. A proliferação da íntima e a remodelação patológica progridem resultando em redução na complacência e aumento da elastância da vasculatura pulmonar (44, 51). Em conjunto, o aumento da elastância e diminuição da complacência contribuem para a redução do fluxo sanguíneo para a região pulmonar, e conseqüentemente da oxigenação tecidual e da qualidade de vida.

6 MECÂNICA RESPIRATÓRIA

A ventilação normal é uma atividade cíclica que ocorre devido aos gradientes de pressão, criados pela expansão do tórax e possui duas fases: inspiração e expiração. Entre os conceitos fundamentais para a compreensão da mecânica respiratória está a complacência pulmonar (CP). A CP representa a capacidade de variar o volume pulmonar para cada variação de pressão, medindo a distensibilidade pulmonar. Já a elastância é a propriedade de resistir à deformação, ou seja, o inverso da complacência (52).

As variações de volume são maiores do que as variações de pressão, então a complacência é basicamente volume-dependente (53). Em outras palavras, a complacência é a relação do volume de gás inspirado pela quantidade de esforço respiratório. O esforço é medido como a quantidade de pressão criada no pulmão, ou no espaço pleural, quando os músculos inspiratórios estão contraídos. Existe uma relação direta entre a complacência e o volume, e uma relação inversa com a pressão. Em última instância, uma redução da complacência significa uma redução na capacidade inspiratória, ou um maior gasto de energia para executar o mesmo trabalho.

A resistência dos pulmões e do tórax constituem a carga contra a qual os músculos respiratórios devem trabalhar para ventilar os pulmões. Em pulmões saudáveis, esse trabalho é mínimo e executado durante a inspiração, pois a expiração é uma manobra passiva no repouso.

O desequilíbrio da ventilação e perfusão, que geralmente ocorre na HAP, pode resultar em hipoxemia, limitando severamente a capacidade do indivíduo para exercer suas atividades diárias. Na HAP, a complacência pulmonar normalmente está reduzida em consequência da presença de inflamação alveolar, fibrose ou mesmo neoplasias nos alvéolos (52).

Pacientes com HAP podem apresentar fluxos expiratórios reduzidos com baixos volumes pulmonares durante a espirometria (54-56). Além disso, a fraqueza muscular inspiratória é um importante determinante da dispneia, um sintoma que afeta a maioria dos pacientes com HAP (57, 58).

De fato, a HAP está associada a certo número de alterações relacionadas a desordens pulmonares, incluindo as restritivas, as alterações obstrutivas, reduzida

capacidade de difusão do monóxido de carbono (59, 60), alteração da relação ventilação/perfusão (61, 62) e redução da força muscular respiratória, principalmente inspiratória (58, 63-65). Essas desordens estão associadas com a congestão pulmonar, edema (66) e correlacionam-se com a capacidade ao exercício reduzida e a uma pior classe funcional na HAP (67). Ainda, essas desordens pulmonares podem contribuir para uma baixa complacência pulmonar e uma elevada elastância pulmonar.

Além disso, a dispneia grave e a fadiga aumentam o trabalho do VD, que excedem a sua capacidade de bombear sangue e contribuem para o edema pulmonar e para a insuficiência ventricular direita de maneira progressiva, demonstrando dessa forma a interação entre os dois sistemas: o sistema cardiovascular e o respiratório (68-70).

De acordo com a literatura, em modelo animal de IC motivada pelo infarto agudo do miocárdio, ocorre um aumento da elastância do sistema respiratório. O estudo também demonstra que o treinamento muscular inspiratório é capaz de restaurar a elastância, demonstrando que o treinamento foi benéfico nesse modelo de IC, uma vez que restabelece pelo menos parcialmente a capacidade inspiratória (71).

No entanto, até o momento não encontramos nenhum estudo avaliando a mecânica respiratória na HAP, especialmente associada ao efeito do exercício sobre este importante parâmetro de funcionamento do sistema respiratório.

Estudos anteriores demonstraram que existe uma redução da complacência pulmonar em diversas patologias, como por exemplo, na alveolite fibrosante, devido à redução do número de alvéolos funcionais e na IC (72-75). Sendo assim, a redução na complacência pulmonar está associada a patologias, e, acreditamos que o exercício, por aumentar o volume de ar e melhorar a relação ventilação/perfusão, possa melhorar a mecânica respiratória no modelo animal de HAP.

O nosso trabalho é o primeiro que tem como objetivo avaliar a mecânica respiratória no modelo animal de HAP, e verificar se o exercício de baixa e/ou moderada intensidade serão capazes de melhorar a mecânica respiratória na HAP.

7 JUSTIFICATIVA E OBJETIVOS

O exercício pode modular as respostas hemodinâmicas, gerando estímulo para o remodelamento estrutural da vasculatura, onde ocorre um aumento na velocidade de fluxo através do vaso, aumentando a tensão de cisalhamento. Isso é um estímulo importante para o alargamento de vasos condutores, que gera uma resposta endotélio-dependente, onde o alargamento é dependente de óxido nítrico. Além disso, o exercício leva ao processo de angiogênese, que requer a proliferação de células endoteliais. O fator de crescimento vascular endotelial (VEGF) parece ser um elemento importante na formação de novos vasos (76). Sabendo disso, e considerando a interação entre o sistema respiratório e cardiovascular, pretendemos estudar o efeito de dois protocolos de exercício sobre a pressão no VD e sobre a mecânica respiratória. Pretendemos não só contribuir com o conhecimento dessa patologia, mas também identificar formas alternativas para tratá-la.

Embora a concepção de que a prática de exercício não seja benéfica na HAP esteja sendo contestada nos últimos tempos, não há dúvidas de que deve ser adotada somente mediante uma avaliação criteriosa, considerando suas vantagens e desvantagens, além de avaliar quais os indivíduos que respondem melhor à adaptação ao exercício.

De fato, mesmo em doenças graves como a HAP (16, 77), e naquelas com falência cardíaca esquerda (78), o exercício físico pode melhorar a função endotelial e a qualidade de vida dos pacientes. Dessa forma, dependendo do protocolo utilizado, os riscos podem ser minimizados, e sua recomendação permitida, mesmo em casos mais graves. Sendo assim, o estabelecimento de um protocolo de exercício que estimule os efeitos desejáveis e promotores de bem estar, pode melhorar a qualidade de vida e a sobrevida dos pacientes com HAP. Segundo Galie e colaboradores (2009), o exercício físico é considerado um complemento promissor para o tratamento medicamentoso, mas seu papel exato no manejo da HAP permanece obscuro (19).

Tendo em vista a importância do bom funcionamento respiratório e da redução da pressão no VD verificada ao longo dessa revisão da literatura, e ainda as altas taxas de mortalidade na HAP e a associação do exercício na função hemodinâmica e na mecânica respiratória, a busca de alternativas não

farmacológicas para complementar o tratamento convencional se torna um importante desafio. Na tentativa da busca de tratamentos complementares, a literatura demonstra que o exercício possui um grande potencial em contribuir para a melhora da qualidade de vida e sobrevida na HAP, no entanto ainda se fazem necessários mais estudos que tenham como objetivo, elucidar os efeitos benéficos do exercício aeróbico, a segurança do mesmo, e tentar identificar as intensidades e qual classificação da doença teriam maior benefício.

Dessa forma, o objetivo desse estudo é avaliar os efeitos de duas intensidades de exercício físico aeróbico sobre o sistema cardiovascular e respiratório, e contribuir para o desenvolvimento de abordagem terapêutica não farmacológica para o tratamento da HAP. Esse estudo pode se somar a outros, e apontar possíveis benefícios do exercício físico também para a mecânica respiratória e para a redução da pressão no VD. A confirmação da nossa hipótese pode contribuir para a melhor qualidade de vida e maior sobrevida para aqueles pacientes que aderirem à prática orientada de exercício físico regular.

Sendo assim, os objetivos específicos desse trabalho são:

1. Avaliar o efeito de duas intensidades de treinamento aeróbico no modelo animal de HAP por MCT sobre o funcionamento respiratório, através da mecânica respiratória, e sobre o sistema cardiovascular, através da mensuração da pressão sistólica e diastólica no ventrículo direito.
2. Identificar qual intensidade de treinamento aeróbico que promova melhor benefício sobre as medidas dos dois sistemas e que, no futuro, melhore a qualidade de vida e sobrevida com o menor risco a saúde dos pacientes com HAP.

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ARTIGO EM INGLÊS

Low intensity of aerobic exercise improves hemodynamic function and respiratory mechanics in rats with pulmonary arterial hypertension

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**LOW INTENSITY OF AEROBIC EXERCISE IMPROVES HEMODYNAMIC
FUNCTION AND RESPIRATORY MECHANICS IN RATS WITH PULMONARY
ARTERIAL HYPERTENSION**

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Running Title: Aerobic Exercise in Pulmonary Arterial Hypertension

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ABSTRACT

Aerobic exercise has been beneficial in patients with Pulmonary Arterial Hypertension (PAH), improving dyspnea and increasing ventilatory muscle strength. On the other hand, it is not very well known which intensity of exercise has better effects on respiratory mechanics and hemodynamic function and less risk to PAH patients. PAH was induced in Wistar rats by a single dose of monocrotaline (50mg/kg; s.c.) in three different groups: PAH-sedentary rats (PAH-S; n=5) and exercised rats in a treadmill for three weeks (5x/week/50min) at low intensity (PAH-L, 40% of maximum speed in incremental test; n=6) and moderate intensity of exercise-training (PAH-M, 60% of the maximum speed in incremental test; n=8). After 3 weeks, rats were tracheostomized to study the respiratory mechanics (FlexiVent) and catheterized for hemodynamic evaluation. Data were compared by one-way ANOVA test followed by Student-Newman-Keuls test ($P<0.05$). Exercise in both intensities significantly decreased ($P<0.05$) right ventricular systolic pressure elevation induced by monocrotaline (RVSP) (PAH-S= 40 ± 9.6 ; PAH-L= 25 ± 2.6 ; PAH-M= 29 ± 5.1 ; mmHg) compared to PAH-S. The quasi-static elastance was significantly lower in PAH-L *versus* PAH-M group (0.71 ± 0.05 vs. 0.98 ± 0.16 ; cmH₂O/mL), whereas the respiratory elastance was lower in the PAH-L *versus* PAH-S group (1.47 ± 0.14 vs. 1.91 ± 0.31 ; cmH₂O/mL). Quasi-static compliance was significantly higher in PAH-L *versus* PAH-S (1.40 ± 0.10 vs. 1.17 ± 0.14 ; mL/cmH₂O) and lower in PAH-M *versus* PAH-L (1.05 ± 0.16 vs. 1.47 ± 0.14 ; mL/cmH₂O). The respiratory system compliance was higher in PAH-L either when compared to the sedentary and moderate groups (PAH-L= 0.68 ± 0.06 vs. PAH-S= 0.53 ± 0.09 , and vs. PAH-M= 0.57 ± 0.05 ; mL/cmH₂O, $P<0.05$). Collectively, these results indicate that low intensity of an aerobic exercise

is effective to decrease the RVSP, which results in a better cardiorespiratory condition during PAH.

Keywords: pulmonary hypertension, monocrotaline, exercise, respiratory mechanics.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare disease with high mortality. The PAH is described by a progressive pulmonary vascular remodeling, which importantly increases right ventricular (RV). It is characterized by a mean pulmonary arterial pressure greater than 25 mmHg at rest and greater than 30 mmHg under exercise (1, 2). Generally, its prevalence is 15 cases per million, being the idiopathic PAH around six cases per million (3). It is characterized by progressive increase in pulmonary vascular resistance, leading to pathological right ventricle (RV) hypertrophy, failure and sudden death (4, 5).

Traditionally, PAH patients are advised to limit physical activity because of the risk of fatal cardiovascular events. On the other hand, regarding to exercise, several studies have been finding the beneficial effects of exercise-training in PAH patients, not only changing the conception that exercise is not safe, but mainly increasing the evidence that exercise could be beneficial.

Mereles et al. (2009) showed great performance, in subjects with PAH, after an exercise-training program. Their findings demonstrated a quality of life improvement after a low intensity-training program (6). Moreover, Man and colleagues (2009) demonstrated that a supervised moderate training program induced a modest improvement in physical performance (7). Souza-Rabbo et al. (2008), working with rats treated with (MCT), showed that a moderate aerobic training reduces RV hypertrophy, mortality and oxidative stress (8).

However, in the progressive PAH, the exercise-training can increase mortality and pulmonary vascular remodeling. These findings indicate that the disease progression and adaptation may be important to show the individual response to exercise-training

in PAH. Indeed, the exercise can be indicated only after a careful evaluation that considers the advantages and disadvantages of an exercise training program for each patient.

Although , it is also important to take into account that, even in severe diseases such as PAH (6, 10), and those associated to left heart failure (11), exercise improves quality of life. Thus, depending on the protocol, the risks can be minimized and an exercise program could be recommended in PAH.

This evidence is clearly demonstrated in the study of Grünig and colleagues (2011). They found that in patients submitted to a low intensity exercise-training associated with drug therapy, there was an increase in survival rate when compared with the advanced pharmacological therapeutic strategies alone (12, 13). These results show the impact of exercise in survival of patients with PAH, and the benefits of adopting pharmacologic and non-pharmacological approaches in this disease.

Currently, exercise-training is being considered a promising complement to pharmacological therapy, but its exact role in the management of PAH remains unclear (14). It is still a challenge to understand which type of exercise could promote better response on cardiac hemodynamic and on respiratory mechanics.

Thus, the objective of this study was to assess the effects of two different intensities of aerobic exercise on the cardiovascular and respiratory systems. Our goal was to contribute for the development of a non-pharmacological therapeutic approach for the treatment of PAH. This study, combined with others, can demonstrate not only the benefits of exercise-training, but also to contribute to establish a less risky exercise protocol that promotes better quality of life and longer survival to patients with PAH.

METHODS

Animals

Eight-week old male Wistar rats were housed in a temperature-controlled room ($23\pm 1^{\circ}\text{C}$), 12:12-hour light/dark cycle with free access to water and food. All procedures were approved by the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) Ethics and Research Committee (protocol number 069/11) and complied with National Institutes of Health guidelines (NIH publication no. 85-23, revised in 1996).

Experimental Pulmonary Hypertension

PAH was induced by a single subcutaneous injection of MCT (50 mg/kg; Sigma-Aldrich®) dissolved in sterile saline.

Study Design and Training Program

The exercise program was based on the protocol described by Fenning et al, (2003) (15). In the first week, all rats were adapted to the treadmill and submitted to the incremental test, described by Pilis et al. (1993) (16), to determine the intensity of exercise-training protocol. In the second week, rats received the single MCT injection and they were assigned to any of the 3 experimental groups: 1- PAH-induced sedentary rats (PAH-S); 2- PAH-induced low intensity exercise-training (PAH-L); 3- PAH-induced moderate intensity exercise-training (PAH-M). During the next three weeks, the trained rats were placed on the treadmill for 50 minutes a day (5 times a week); the moderate ($\approx 60\%$ $\text{VO}_{2\text{max}}$) intensity group ran with a constant speed of

approximately 13.3 m/min (9, 17), and the low ($\approx 40\%$ VO_2max) intensity group 10 m/min. The sedentary group was placed for five minutes inside the treadmill, with no velocity or slope, and kept close to the treadmill until the end of the training period.

At the end of the exercise-training protocol, a second incremental test for functional and capacity evaluation was performed. Two days after the end of exercise training, the cardiovascular and the respiratory mechanic were evaluated.

Assessment of Respiratory Mechanics

The rats were anesthetized with a cocktail, comprising ketamine (80 mg/kg), xylazine (10 mg/kg) and diazepam (0.5 mg/kg). All procedures for respiratory mechanics assessment were according to Jaenisch et al. (2011) (18). Rats were tracheostomized using a rigid-type cannula (2-mm ID) connected to a small animal ventilator (Flexi Vent, SCIREQ®, Montreal, PQ, Canada), for mechanic ventilation. The constant-phase model was fitted as follows: $Z_{rs}(f) = R_{aw} + i2\pi f l_{aw} + [G_{ti} - iH_{ti}] / (2\pi f)^\alpha$, where Z_{rs} , R_{aw} , l_{aw} , G_{ti} , H_{ti} , i is and f are the respiratory system impedance, airway resistance, inertance, tissue resistance, lung elastance, the imaginary unit and frequency, respectively. $\alpha = (2/\pi) \arctan(H_{ti}/G_{ti})$ (19). Quasi-static elastance (E_{st}) reflects the static elastic recoil pressure of the lungs at a given lung volume, and was measured by applying the Pressure-Volume curve technique (the Salazar-Knowles equation) as described by the *flexi Vent* manufacturer.

Cardiovascular evaluation

After evaluating the respiratory mechanics, still under anesthesia, the right ventricular (RV) pressure was measured using a polyethylene catheter inserted into the right descending jugular vein to reach the RV.

To record the data (2000 Hz/sample rate/channel), a polyethylene catheter was connected to a strain-gauge pressure transducer (Strain-Gauge-NarcoBiosystem Miniature Pulse Transducer RP-155, Houston, Texas, USA), coupled to a pressure amplifier (Stemtec®). After tracing stabilization, the RV pressure signal was recorded over 5-min period (CODAS, 1-kHz sampling frequency, Dataq® Instruments, Inc., Akron, OH, USA). The data were analyzed on a beat-to-beat basis to determine, right ventricular systolic pressure (RVSP) and right ventricular diastolic pressure (RVDP). Still under anesthesia, rats were killed by decapitation to collect heart, lungs and liver for posterior evaluations.

Heart Hypertrophy and Pulmonary Congestion

The right ventricle (RV) and left ventricle (LV) were dissected and weighed. The heart (HW/BW), LV (LV/BW) and RV (RV/BW) to body weight ratio were determined. Lungs were dehydrated (80°C/48h) and weighed again to determine the water percentage.

Statistical analysis

First, the data were tested for normal distribution by the Kolmogorov-Smirnov test and analyzed by one way ANOVA, followed by the Student Newman-Keuls *post hoc* test. Pearson's correlation analysis was performed for testing associations. To achieve those purposes, the GraphPad Prism 5 program (GraphPad® Software, San Diego, CA) and Sigma Plot® (Systat Software Inc., San Jose, USA) for Windows were used in the data analysis. Data are presented as mean±SD and a $P < 0.05$ was considered statistically significant.

RESULTS

Morphometric data, Pulmonary and Hepatic Congestion, and Heart

Hypertrophy

Before exercise training, there was no change in body weight and incremental test. The exercise-training did not change body weight, heart hypertrophy, pulmonary and hepatic congestion. On the other hand, the exercise-training significantly increased the speed in the incremental test in both trained groups, compared to sedentary rats (Table 1).

Hemodynamic variables

The low and moderate intensity exercise did induce a significant decreased in RVSP (33%, PAH-L and 26%, PAH-M) and RVDP (45%, PAH-L and 46%, PAH-M), compared to PAH-S group. The heart rate was similar among groups (Table 2).

Respiratory Mechanics

Regarding to respiratory system resistance, there was no significant difference among groups [PAH-S= 0.11 ± 0.07 ; PAH-L= 0.11 ± 0.03 and PAH-M= 0.12 ± 0.04 ; $F(2,17) = 0.077$, $P=0.93$], tissue resistance [PAH-S= 0.49 ± 0.02 ; PAH-L= 0.43 ± 0.06 and PAH-M= 0.46 ± 0.03 ; $F(2,17)= 2.5$, $P=0.13$] and tissue elastance [PAH-S= 1.89 ± 0.36 ; PAH-L= 1.51 ± 0.25 and PAH-M= 1.68 ± 0.19 ; $F(2,17)= 2.32$; $P=0.14$]. In addition, in the PAH-L group, exercise induced a decrease [23%; $F(2,17)=5.0$, $P=0.03$] in the respiratory system elastance (Figure 1.A), and a significant ($P<0.05$) increase [26%; $F(2,17)= 5.3$, $P=0.02$] in the compliance (Figure 1.B) and in the quasi-static compliance [20%; $F(2,17)=5.0$, $P=0.03$], (Figure 1.C) when compared to PAH-S

group. However, the PAH-M group presented a significant ($P<0.05$) increase in the quasi-static elastance [29%; $F(2,17)=3.9$, $P=0.04$], (Figure 1.D) and a decrease in the compliance (15%, Figure 1.B) and in the quasi-static compliance (21 %, Figure 1.C) compared to PAH-L group.

Correlations

There were a significant and negative correlation between the “Incremental Test” and RVSP ($r=-0.502$, $P=0.02$) and RVDP ($r=-0.769$, $P<0.01$) after the exercise training. There were also a significant and positive correlation between RVSP and quasi-static elastance ($r=0.572$, $P=0.03$) and a negative with the quasi-static compliance($r= -0.587$, $P=0.02$).

DISCUSSION

In the present study we demonstrated, for the first time, the effects of low and moderate aerobic exercise intensities over the RV pressure and respiratory mechanics in MCT-PAH. We show that aerobic exercise was well tolerated and induced a significant decreased in RVSP and RVDP. However, only the low intensity group showed an increase in the respiratory compliance and a decrease in the respiratory system elastance. The low intensity exercise showed a better respiratory mechanics response than the moderate exercise.

Handoko et al. (2009) conducted an experimental study to assess the effects of exercise-training in two phenotypes of PAH. They demonstrated that exercise-training increased wet lung weights in the progressive (60 mg/kg of MCT) PAH group, whereas it had no effect on lung weights in the stable PAH group (40 mg/kg of MCT).

They suggest that exercise-training modulates the pulmonary vascular remodeling and that the differences were not likely to be attributed to pulmonary edema (9). In addition Souza-Rabbo et al. (2008), also working with 60mg/kg of MCT, demonstrated that exercise did not prevent the MCT induced lung congestion (8). Collectively, these results underscore the importance of the exercise intensity and the stage of PAH to conclude about the effects of exercise on PAH.

Regarding to the RV hypertrophy, Handoko et al. (2009) (9) did not find differences after four weeks of PAH comparing stable (40 mg/kg of MCT) and progressive (60 mg/kg) MCT-PAH groups. In accordance to Handoko et al., Souza-Rabbo et al. (2008), who had used 60mg/kg of MCT, also showed that four weeks of exercise did not reduce RV hypertrophy (8). On the other hand, similarly to our results, Souza-Rabbo et al. (2008) (8) found a RV hypertrophy attenuation after three weeks of exercise-training.

Considering that MCT treatment is a time and dose-dependent model and that the time of each exercise protocol probably induces different hemodynamic responses, it is reasonable to find those differences among results.

Few studies in animal models showed the effects of exercise-training in PAH. Handoko et al. (2009) was the first group to describe that a moderate intensity of exercise-training did not decrease the RVSP and RVDP. On the other hand, they did show that exercise-training improved capillarization, whereas a trend for the opposite effects was seen in the progressive PAH (9). We did find a reduction in both RVSP and RVDP in PAH-M group. The differences between our and their results could be attributed to the combination of MCT dose and the time of exercise-training.

Another interesting data of our study was the negative correlation found between RVSP and RVDP with the incremental test after exercise. These results

demonstrate that in trained rats, which probably have better aerobic capacity, there was an improvement also in the hemodynamic variables. Indeed, there are data in the literature showing that aerobic exercise modulates the hemodynamic responses by improving the morphology of the arterial vessels and inducing angiogenesis (20).

Normally, during PAH occurs a RV dysfunction, with a pathological remodeling, hypertrophy and with an increase in RVSP. These characteristics have been described in several studies as being related to the severity and prognosis of PAH (21-23) and also that a low stroke volume in the RV, and hence a low RVSP, is a predictor of survival in PAH patients (23) having clinical and social relevance. Thus, any approach that can reduce RVSP could be a promising complement to provide better patients quality of life and decrease the PAH mortality.

Severe dyspnea and fatigue reflect high levels of RV work, contributing to RV ischemia, inflammation, pulmonary edema and progressive failure. These findings demonstrate the interaction of the cardiovascular and respiratory system (21-23). In fact, our results demonstrated a negative correlation between RVSP and the quasi-static complacence, and a positive correlation with quasi-static elastance indicating the close association between the respiratory mechanics and the cardiovascular system. It is also very well established in the literature that compliance is the change in the volume *versus* a pressure, and the elastance represents an inverse association. The higher the elastance higher the stiffer of the small pulmonary arteries wall (24).

Regarding to this specific focus, we have demonstrated, for the first time that the PAH-L group has improved the respiratory mechanics function. Comparing to the sedentary group, the low intensity exercise-training increases the compliance and a decrease the elastance. On the other hand, the PAH-M group showed no difference

in the compliance and also in the elastance *versus* sedentary group. This result suggests that the low intensity of aerobic exercise improves the respiratory mechanics during PAH and that the intensity is crucial to obtain beneficial effects with exercise.

In addition, it was reported in the literature that with the progression of PAH there is an increase in the intimal proliferation and a pathological remodeling (24), contribute for the progression of PAH.

Our data showing the decrease in the elastance after exercise agree with the literature that, in an animal model of heart failure, there was an increase in respiratory system elastance, and that the respiratory muscle training restores this parameter (18).

In addition, Goncalves et al. (2012) also demonstrated the beneficial effects of exercise-training in a model of acute lung injury. Their results showed that a low intensity of aerobic exercise decreases the pulmonary resistance and elastance (25).

However, as far as we research, there was no studies evaluating the effects of two different intensities of exercise-training in the respiratory mechanics in a PAH model. Our work is the first to conclude that, different exercise-training intensities may result in an improvement of RVSP and RVDP. Nevertheless, only the low intensity of exercise improves the respiratory system mechanics, resulting in a better cardiopulmonary interaction in a model of PAH. The hypothesis, which we try to explain why the moderate aerobic exercise did not improved the respiratory mechanics, is that during a higher level of exercise the pressure at the pulmonary arteries increases, and it contributes to a worse pulmonary and cardiovascular remodeling.

Our findings clearly indicate that exercise, at low intensity, is a promising non-

pharmacological approach for treating PAH and, which could contribute to others studies and provide better patient's quality of life and a reduction in PAH mortality.

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DISCLOSURES

The authors have no financial relationship with any commercial entity with an interest in the subject of this paper.

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FIGURE LEGENDS

Figure 1.

Respiratory Mechanics. Values are means $\pm$ standard deviation; One way ANOVA and Student Newman-Keuls post hoc test. PAH-S (n=5) = pulmonary arterial hypertension sedentary rats; PAH-L (n=6)= pulmonary arterial hypertension low intensity; PAH-M (n=8)= pulmonary arterial hypertension moderate intensity. **Panel A:** Ers= Respiratory system elastance; *P<0.05 vs. PAH-L; **Panel B:** C = Respiratory system compliance; *P<0.05 vs. PAH-L; **Panel C:** Cst= Quasi-static compliance; *P<0.05 vs. PAH-L; **Panel D:** Est= Quasi-static elastance; #P<0.05 PAH-L vs. PAH-M.

Figure 1.

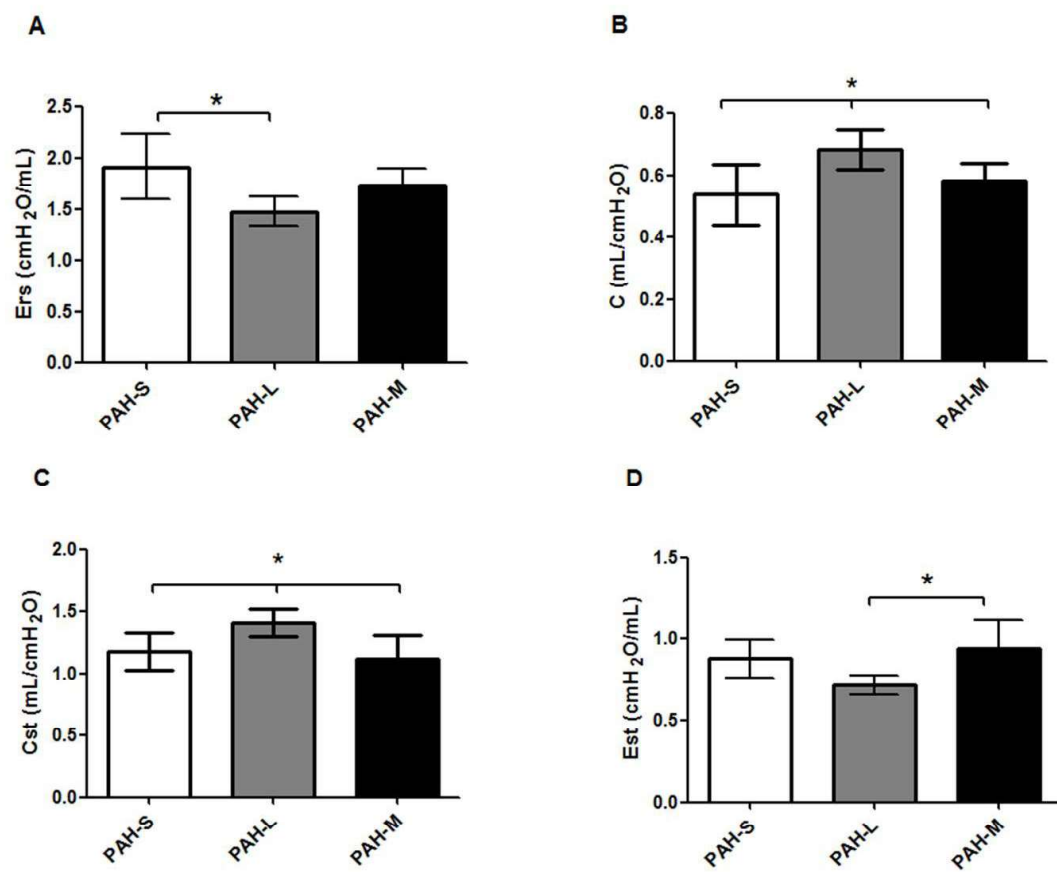


Table 1. *Morphometric data, Pulmonary, Hepatic Congestion and Heart hypertrophy index in all experimental groups*

Data	PAH-S (n=5)	PAH-L (n=6)	PAH-M (n=8)
Body Weight Before (g)	145±21	128±16	138±14
Body Weight After (g)	331±23	320±29	302±29
IT Before (km/h)	1.50±0.21	1.40±0.15	1.42±0.14
IT After (km/h)	1.14±0.39	1.85±0.22*	1.83±0.19*
Pulmonary Congestion (%)	82±5.1	83±2.4	81±1.9
Hepatic Congestion (%)	73±1.4	73±1.8	72±1.3
H/BW (mg/g)	2.54±0.11	2.40±0.09	2.57±0.22
H/T (mg/mm)	23±2.5	21±2.3	21±1.7
LV/BW (mg/g)	2.00±0.07	1.96±0.10	2.06±0.17
LV/T (mg/mm)	18±2.0	17±1.8	17±1.4
RV/BW (mg/g)	0.56±0.09	0.53±0.05	0.54±0.11
RV/T (mg/mm)	5.04±0.71	4.45±0.67	4.35±0.84

PAH-S= pulmonary arterial hypertension sedentary rats; PAH-L= pulmonary arterial hypertension low intensity; PAH-M= pulmonary arterial hypertension moderate intensity. IT= Incremental test; H/BW= Heart weight to body weight; H/T= Heart weight to tibia measure; LV/BW= Left ventricle weight to body weight; LV/T= left ventricle weight to tibia measure; RV/BW=Right ventricle to body weight; RV/T=Right ventricle to tibia measure. *P<0.05 vs. PAH-S

Table 2. *Hemodynamic variables in all experimental groups*

Variables	PAH-S (n=5)	PAH-L (n=6)	PAH-M (n=8)
RVSP (mmHg)	40±8.6	25±2.6*	29±5.1*
RVDP (mmHg)	7.4±1.5	3.5±0.2*	3.9±0.8*
HR (bpm)	227±50	188±35	248±64

PAH-S = pulmonary arterial hypertension sedentary rats; PAH-L = pulmonary arterial hypertension low intensity; PAH-M = pulmonary arterial hypertension moderate intensity. RVSP = Right Ventricular Systolic Pressure; RVDP = Right Ventricular Diastolic Pressure; HR = heart rate in beats per minute; *P<0.05 vs. PAH-S. Values represent means ± standard deviation.

ANEXO I



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A Comissão de Ética no uso de Animais, analisou o Projeto:

Projeto: 11-037

Versão do Projeto:

Versão do TCLE:

Pesquisadores:

KATYA VIANNA RIGATTO

DENIELLI DA SILVA GONÇALVES BÓS

Título: INFLUÊNCIA DA INTENSIDADE DE EXERCÍCIO FÍSICO
SOBRE O QUIMIORREFLEXO DA HIPERTENSÃO PULMONAR.

Este projeto foi aprovado em seus aspectos éticos e metodológicos. Todo e qualquer alteração do projeto, assim com eventos adversos graves, deverão ser comunicados a esta CEUA.

Porto Alegre, 13 de junho de 2011.

Pedro R. T. Romão
Coordenador CEUA/UFSCPA

ANEXO II

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Keywords

Include three to five words or short phrases, relevant to the article, that do not appear in the title or running head.

Introduction

Provide a brief overview of the scope and relevance of the study, especially with regard to previous advancements in related fields.

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(Sometimes called "Experimental Procedures"). Describe techniques, cell/animal models used, and lists of reagents, chemicals, and equipment, as well as the names of manufacturers and suppliers, including URLs for those supplies obtained online, so that your study can be most easily replicated by others. Also in this section, describe the statistical methods that were used to evaluate the data. If clinical trials were used, a statement of registration is required; also, for all investigations involving humans or animals, a statement of protocol approval from an IRB or IACUC, or an equivalent statement, must be included. **All animal or human studies must contain an explicit statement that the protocols were submitted to, and approved by, an institutional review board or committee or that the protocols were performed under a license obtained from such a committee, board, or governing office.** (see *Use of Humans and/or Animals in Experiments* above). See *Abbreviations, Symbols, and Terminology* (above) for style information.

Results

Provide the experimental data and results as well as the particular statistical significance of the data. APS has published an editorial on the use

of statistics (<http://physiolgenomics.physiology.org/cgi/content/full/18/3/249>), and authors are encouraged to consult it.

Discussion

(Sometimes combined with the results in a section called "Results and Discussion"). Explain your interpretation of the data, especially compared with previously published material cited in the References.

Acknowledgments

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[journals/styles.htm](#).

Examples

Journal Articles

1. Villalobos AR, Parmelee JT, Renfro JL. Choline uptake across the ventricular membrane of neonate rat choroid plexus. *Am J Physiol Cell Physiol* 276: C1288–C1296, 1999.

Book References

1. Pollock DM. Endothelin receptor subtypes and tissue distribution. In: *Endothelin Molecular Biology, Physiology, and Pathology*, edited by Highsmith RF. Totowa, NJ: Humana, 1998.

APS Handbook of Physiology Series

Large textbooks require very specific citation information. For example, the APS Handbooks series contains a huge amount of information, and the inclusion in the citation of the section, volume, part, and chapter is essential to aid the reader in finding the information quickly (please note that the APS chooses not to list editors for the APS Handbooks).

1. Kaufman MP, Forster HV. Reflexes controlling circulatory, ventilatory, and airway responses to exercise. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems. Control of Respiratory and Cardiovascular Systems*. Bethesda, MD: Am. Physiol. Soc., 1996, sect. 12, pt. II, chap. 10, p. 381–447.

For an exhaustive set of examples for properly citing various types of publications, including articles published on the web, technical documents, congress proceedings, articles with errata/corrigenda, translations, and articles with large groups of authors, go to <http://www.the-aps.org/publications/authorinfo/examplerefs.htm>.

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1. Scarafia LE, Winter A, Swinney DC. Quantitative expression analysis of the cellular specificity of HECT-domain ubiquitin E3 ligases. *Physiol Genomics* (April 26, 2001). doi:10.1152/physiolgenomics.00075.2001.

However, once this article has reached its final stage of publication, it will be cited with its new publication data, as follows:

1. Scarafia LE, Winter A, Swinney DC. Quantitative expression analysis of the cellular specificity of HECT-domain ubiquitin E3 ligases. *Physiol Genomics* 4: 147–153, 2001. First published April 26, 2001; doi:10.1152/physiolgenomics.00075.2001.

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- Each table should have a brief title; explanatory notes should be in the legend, not in the title.
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Mathematical aspects of articles normally should be addressed to the many readers of the Journal who are not mathematicians. The presentation should include the mathematical strategy, the assumptions on which the mathematics are based, and a summary of the meaning of the final mathematical statement and its limitations.

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Mathematical equations should be simplified as much as possible and carefully checked.

Use the slant line (/) for simple fractions $(a + b)/(x + y)$ in the text rather than the built-up fraction

$$\frac{a + b}{x + y},$$

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Use subscripts or superscripts wherever feasible and appropriate, to simplify the equations.

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Presentation of the model(s) must be sufficiently clear to allow physiologists with limited experience in modeling to follow the model development, limitations, and physiological relevance. Assumptions concerning the importance of physiological processes included in the model should be clearly stated.

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