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Avaliação da Modulação Simpatovagal e da Função Endotelial Periférica após Treinamento Muscular Inspiratório em Pacientes com Hipertensão Pulmonar dos Grupos I e IV.

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Orientador: Dra. Katya Rigatto

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RESUMO

INTRODUÇÃO: Hipertensão arterial pulmonar (PAH) e hipertensão pulmonar associada ao tromboembolismo crônico (CTEPH) são caracterizadas por desbalançosimpatovagal, disfunção endotelial, aumento da pressão na artéria pulmonar e sobrecarga cardíaca direita. Pacientes portadores destas doenças apresentam acentuada dispnéia e fadiga, que diminuem a capacidade funcional. Neste sentido, o treinamento da musculatura inspiratória (IMT) pode melhorar a respiração e diminuir as restrições funcionais desses pacientes. Dessa forma, os objetivos do presente trabalho foram verificar se há correlação entre o balanço simpatovagal, a disfunção endotelial periférica e a capacidade funcional dos pacientes com PH e avaliar a resposta destes desfechos após IMT. MÉTODOS: Pacientes com PAH e CTEPH realizaram IMT diariamente por 8 semanas.Antes e após o treinamento, foram realizados ultrassonografia da artéria braquial, eletrocardiograma e avaliação da capacidade funcional e da força muscular inspiratória.RESULTADOS: A avaliação basal dos pacientes apresentou modulação parassimpática reduzida, que se correlacionou negativamente com a capacidade funcional. No entanto, o IMT promoveu aumento da modulação parassimpática e diminuição da simpática, com consequente melhora do balanço simpatovagal. Com relação à função endotelial, os pacientes apresentaram provável disfunção endotelial periférica e não houve melhora significativa nestes resultados após o treinamento. CONCLUSÃO: Não houve correlação entre a função endotelial periférica com a capacidade funcional ou modulação simpatovagal. No entanto, a modulação parassimpática reduzida e consequente desbalançosimpatovagal correlacionaram-se, de maneira inversa, com a capacidade funcional na avaliação basal. Após o IMT, apesar de não haver diferença na função endotelial, houve melhora significativa no balanço simpatovagal, provavelmente reduzindo o risco cardiovascular nestes pacientes.

PALAVRAS-CHAVE: Hipertensão arterial pulmonar. Hipertensão pulmonar associada ao tromboembolismo crônico. Balanço simpatovagal. Funçãoendotelial periférica. Treinamento muscular inspiratório.

ABSTRACT

BACKGROUND: Pulmonary arterial hypertension (PAH) and pulmonary hypertension associated with chronic thromboembolism (CTEPH) are characterized by sympatovagal imbalance, endothelial dysfunction, pulmonary arterial pressure increase and right heart overload. Patients with these diseases have marked dyspnea and fatigue, which reduce their functional capacity. In this way, the inspiratory muscle training (IMT) can improve the breathing and decrease the functional restrictions of these patients. Thus, the objectives of the present study were to verify if there is correlation among the sympatovagal balance, the peripheral endothelial dysfunction and the functional capacity of PH patients and to evaluate the response of these outcomes after IMT. METHODS: PAH and CTEPH patients performed IMT daily for 8 weeks. Before and after the training, brachial artery ultrasonography, electrocardiogram, and functional capacity and inspiratory muscle strength assessment were performed. RESULTS: The baseline evaluation presented reduced parasympathetic modulation, which was correlated negatively with functional capacity. However, IMT promoted parasympathetic modulation increase and sympathetic decrease, with consequent sympatovagal balanceimprovement. Regarding to endothelial function, the patients presented probable peripheral endothelial dysfunction and there was no significant improvement in these results after training. CONCLUSION: There was no correlation between peripheral endothelial function and functional capacity or simpatovagal modulation. However, reduced parasympathetic modulation and consequent sympathovagal imbalance were correlated, in a different way, with functional capacity in baseline assessment. After IMT, although there was no difference in endothelial function, there was a significant improvement in the sympatovagal balance, probably reducing the cardiovascular risk in these patients.

KEYWORDS: Pulmonary arterial hypertension. Pulmonary hypertension associated with chronic thromboembolism. Sympathovagal balance. Peripheral endothelial function. Inspiratory muscle training.

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LISTA DE ABREVIATURAS

ACh	Acetilcolina
CPET	Teste de esforço cardiopulmonar
CTEPH	Hipertensão pulmonar associada ao tromboembolismo crônico
ECG	Eletrocardiograma
ERA	Antagonistas dos receptores de endotelina
FMD	Dilatação mediada por fluxo
HF	Alta frequência
HR	Frequeência cardíaca
HRV	Variabilidade da frequência cardíaca
IMT	Treinamento muscular inspiratório
LF	Baixa frequência
mPAP	Pressão média da artéria pulmonar
NO	Óxido nítrico
PAH	Hipertensão arterial pulmonar
PDE5i	Inibidores da fosfodiesterase 5
PH	Hipertensão Pulmonar
PVR	Resistência vascular pulmonar
RHC	Cateterismo cardíaco direito
RV	Ventrículo direito
sGCs	Estimuladores solúveis da guanilatociclase
SPNS	Sistema nervoso simpático e parassimpático
6MWT	Teste de caminhada de seis minutos
VLF	Muito baixa frequência
WHO	Organização Mundial da Saúde

SUMÁRIO

1 REVISÃO DE LITERATURA

1.1 Hipertensão Pulmonar

Hipertensão Pulmonar (PH) é uma condição causada por alterações hemodinâmicas e fisiopatológicas que resultam em elevaçãodos níveis pressóricos na circulação pulmonar. É definida por pressão média na artéria pulmonar (mPAP) igual ou superior a 25mmHg em repouso, aferida por cateterismo cardíaco direito (RHC)(1).

A PH é classificada clinicamente em cinco grupos com a finalidade de categorizar múltiplas condições de acordo com suas apresentações clínicas similares, fisiopatologia, características hemodinâmicas e estratégias de tratamento (2, 3). São eles: Grupo I – Hipertensão Arterial Pulmonar; Grupo II - Hipertensão pulmonar associada à doença cardíaca esquerda; Grupo III - Hipertensão pulmonar associada a doenças pulmonares e/ou hipóxia; Grupo IV - Hipertensão pulmonar associada ao tromboembolismo crônico e outras obstruções da artéria pulmonar e Grupo 5 - Hipertensão pulmonar com mecanismos multifatoriais indefinidos. No presente trabalho, foram selecionados pacientes dos grupos I e IV da PH devido a características fisiopatológicas semelhantes, conforme descrito adiante neste referencial.

O grupo I, chamado de Hipertensão Arterial Pulmonar (PAH), é hemodinamicamente definido por mPAP ≥25mmHg, pressão de oclusão da artéria pulmonar < 15mmHg e resistência vascular pulmonar (PVR) ≥ 3 unidades de Wood (4). De acordo com estudos europeus, a incidência da PAH está estimada em torno de 1,1 a15 casos/milhão(5, 6). No Brasil, existem poucos estudos representativos da população, mas uma publicação de dados epidemiológicos da região sudeste relata a prevalência de 123pacientes com PAH em dois centros brasileiros da especialidade(7). Neste contexto, salientamos a

importância da realização de mais estudos com esta população no nosso país, visando melhorar o conhecimento tanto epidemiológico quanto fisiopatológico desta doença.

Entre as alterações presentes na PAH temos proliferação celular, remodelamento tecidual, vasoconstrição, inflamação e trombose, levando a um aumento gradual da PVR (8, 9). A alta PVR leva ao aumento da pós-carga no ventrículo direito (RV) e gera a necessidade de ação compensatória muscular, com consequente hipertrofia ventricular direita. Essa tentativa de adaptação cardíaca pode ser efetiva, caracterizada por hipertrofia concêntrica e manutenção da fração de ejeção e débito cardíaco; ou pode haver uma descompensação, com afinamento e dilatação da parede do músculo cardíaco direito e redução da fração de ejeção, trazendo serias consequências para a saúde do paciente(4, 10).Com o agravamento do quadro, as consequências invariavelmente são falência ventricular direita e morte súbita(11), que justificam a grande preocupação da comunidade cientifica em encontrar novas alternativas de diagnóstico e tratamento para esta doença.Embora o aumento da pós-carga seja o primeiro desencadeante do remodelamento ventricular direito, sinalização neurohormonal, estresse oxidativo, inflamação, isquemia e morte celular podem contribuir para o desenvolvimento da dilatação e insuficiência(12).

A conduta terapêutica na PAH divide-se basicamente em 3 áreas principais. A primeira área engloba medidas gerais, que compreendem reabilitação física e pulmonar, terapia de suporte, entre outros. A segunda área inclui todos os fármacos vasodilatadores aprovados, como antagonistas de receptores da endotelina (ERA – Bosentana, Ambrisentana, Macitentana), análogos de prostaciclinas (Iloprosta, Teprostinila, Epoprostenol, Beraprost), inibidores da fosfodiesterase 5 (PDE-5i – Sildenafila, Tadalafila) ou estimuladores solúveis da guanilatociclase (sGCs – Riociguate). Em conjunto, esses medicamentos vasodilatadores tratam a doença por atuação no endotélio

vascular, aumentando a capacidade de transporte sanguíneo nos pulmões e promovem melhora na tolerância ao exercício, hemodinâmica pulmonar e qualidade de vida. A terceira área corresponde ao manejo da resposta clínica, quando inadequada, e abrange combinações de fármacos aprovados e procedimentos intervencionistas adicionais, como a septostomia e o transplante pulmonar(3).

O grupo IV compreende uma causa de PH potencialmente curável, a hipertensão pulmonar associada ao tromboembolismo crônico (CTEPH). Os sinais clínicos e a definição hemodinâmica são os mesmos da PAH, entretanto, este grupo também apresenta sinais específicos encontrados em exames de imagem que caracterizam a doença (3). De acordo com o Registro espanhol de PH, a prevalência e a incidência de CTEPH são 3,2 e 0,9 casos por milhão por ano, respectivamente(13). Dentre os fatores etiológicos atuantes nesta doença, encontram-se anormalidades na coagulação (deficiência de antitrombina, proteína C e proteína S, e mutação no fator V, entre outros) e tromboses não resolvidas (14).

A fisiopatologia deste grupo caracteriza-se pela presença inicial de um trombo venoso, que irá torna-se um embolismo pulmonar agudo. A resolução do tromboembolismo agudo impede a instalação da doença. No entanto, quando o tromboembolismo cronifica, ocorre o desenvolvimento de estenoses e oclusões fibróticas na vasculatura pulmonar. Estas obstruções persistentes resultam no redirecionamento do fluxo sanguíneo para outros vasos não-ocluídos do pulmão, levando a ao aumento da pressão e do estresse de cisalhamento e à liberação de fatores inflamatórios (14). Dessa forma, desenvolve-se, nas áreas não ocluídas, a mesma arteriopatia característica do grupo 1, PAH, com disfunção endotelial, remodelamento vascular e formação das lesões plexiformes que, em conjunto, vão levar ao aumento da PVR e RHF. Estas alterações

endoteliais explicam a natureza progressiva da doença, mesmo na ausência de novos trombos(15).

O tratamento para CTEPH pode ser cirúrgico, através da cirurgia de tromboendarterectomia nos caso de lesões proximais, ou medicamentoso, para os casos de lesões distais com impossibilidade de remoção cirúrgica. Nestes casos, a terapia pode ser realizada com as mesmas medicações vasodilatadoras utilizadas para o tratamento da PAH. No entanto, o único medicamento aprovado diretamente para o tratamento do CTEH é o Riociguate, queaumenta a biodisponibilidadede doóxido nítrico (NO)(16).

Os sintomas da PH são pouco específicos e estão relacionados com o prejuízo das trocas gasosas e disfunção ventricular direita. São exemplos dispneia, fadiga, fraqueza, angina, síncope, retenção hídrica, entre outros, que limitam as atividades diárias e a qualidade de vida dos pacientes (3). O grau de intolerância ao esforço é quantificado de acordo com a classificação de insuficiência cardíaca da Organização Mundial da Saúde (WHO), que reflete a gravidade da PH e o impacto que a doença causa na vida do paciente em termos de atividades diárias e sintomas(17). Esta classificação é dividida em quatro classes, sendo a classe I a menos grave e a classe IV a mais avançada (Tabela 1).

Tabela 1: Classificação funcional da HP de acordo com a WHO

CLASSE I	Pacientes com PH, mas sem limitação das atividades físicas. Atividades físicas habituais não causam dispneia ou fadiga excessiva, dor torácica ou pré-síncope.
CLASSE II	Pacientes com PH que resulta em discreta limitação das atividades físicas. Estes pacientes estão confortáveis ao repouso, mas atividades físicas habituais causam dispneia ou fadiga excessiva, dor torácica ou pré-síncope.
CLASSE III	Pacientes com PH que resulta em relevante limitação das atividades físicas. Estes pacientes são confortáveis ao repouso, mas esforços menores que as atividades físicas habituais causam dispneia ou fadiga excessiva, dor torácica ou pré-síncope.
CLASSE IV	Pacientes com PH que resulta em incapacidade para realizar qualquer atividade física sem sintomas. Estes pacientes manifestam sinais de falência ventricular direita. Dispneia ou fadiga podem estar presentes ao repouso, e o desconforto aumenta com qualquer esforço feito.

HP = hipertensão pulmonar; WHO = Organização Mundial da Saúde.Tabela adaptada de(17)

1.2 Capacidade Funcional

Recomenda-se, para melhor estratificação de risco e manejo da doença, uma avaliação regular dos pacientes. Neste contexto, a avaliação da capacidade funcional é de especial importância, pois auxilia na determinação da severidade da doença, resposta ao tratamento e predição de sobrevida (3). A capacidade funcional está intimamente relacionada com a fração de ejeção do RV; dessa forma, um RV insuficiente apresenta dificuldade em adequar o fluxo pulmonar à demanda de oxigênio, uma vez que, além de ele ser fraco, também enfrenta uma pós carga aumentada (2). Em contrapartida, RV com função preservada está associado a melhor tolerância ao exercício e sobrevida(18).

O teste de caminhada de seis minutos (6MWT) e o teste de esforço cardiopulmonar (CPET), além de serem exames não-invasivos, fornecem informações importantes sobre a capacidade funcional, bem como sobre a troca de gases, a eficácia ventilatória e função cardíaca durante o exercício(2, 19) O 6MWT reflete uma avaliação submáxima, é bem tolerado pela maioria dos pacientes, possui baixo custo e apresenta forte e independente associação com mortalidade (19). Já o CPET é um teste máximo que avalia as mudanças fisiológicas associadas ao exercício e também apresenta importante valor prognóstico. O consumo de oxigênio e a pressão sistólica em pico são fortes preditores de sobrevida em pacientes com PAH (20). O importante valor clínico dos resultados apresentados por estes exames, para avaliação da capacidade funcional dos pacientes com PH, foi considerado para a inserção destas análises nos sujeitos participantes deste estudo.

1.3 O endotélio vascular

As células endoteliais são responsáveis pela regulação da homeostase vascular e servem como interface entre o sangue e o tecido. O endotélio regula o tônus vascular através da produção de vasoconstritores e vasodilatadores, controla a fluidez e a coagulação do sangue através de fatores que regulam a atividade das plaquetas, a cascata de coagulação e o sistema fibrinolítico e, por fim, é responsável pela produção de citocinas e moléculas de adesão que atuam no processo inflamatório (21).

A disfunção endotelial é certamente um ponto crucial no desenvolvimento de condições patológicas, como aterosclerose (22) e hipertensão pulmonar (4, 8), entre outras. Múltiplas vias estão relacionadas com o desenvolvimento da PH, incluindo aquelas em níveis moleculares e genéticos e aquelas relacionadoas às células musculares lisas, endoteliais e da adventícia(10). No endotélio pulmonar na PAH, inicialmente ocorre o espessamento da íntima por proliferação e migração celular. Após, as camadas média e adventícia também sofrem alteração por hipertrofia e acúmulo de fibroblastos. Quando o processo obstrutivo vascular já está instalado, ocorre hipertrofia e hiperplasia das células endoteliais e a formação de lesões plexiformes, que são uma proliferação monoclonal de células endoteliais formando múltiplos canais no interior de arteríolas, cercados de células musculares lisas, matriz extracelular e miofibroblastos. Em paralelo com o remodelamento vascular, ocorrem também microtromboses nas artérias pulmonares geralmente causadas por coagulação intravascular, disfunção plaquetária e lançamento de mediadores constritores e pró-coagulantes pelas células endoteliais e liberação de fatores inflamatórios (4, 8).

Ainda, a fisiopatologia da PAH envolve desbalanço na produção de mediadores que podem produzir vasodilatação, como o NO e as prostaciclinas, ou vasoconstrição (23). As células endoteliais aumentam o diâmetro vascular liberando NO, que é produzido nas células endoteliais pela NO sintase a partir de estímulos como a liberação de acetilcolina

(ACh), estímulos mecânicos, como o estresse de cisalhamento do sangue, e a disponibilidade da L-arginina, que é o precursor do NO (24, 25). Ele é o principal mediador da dilatação mediada por fluxo (FMD), fenômeno que ocorre em resposta ao aumento do fluxo sanguíneo em determinado vaso, ou estresse de cisalhamento (26). A deficiência doNO é característica em doenças cardiovasculares e geralmente é preditivo de desfechos clínicos reservados (22, 27). Devido a sua ação vasodilatadora, o NO é bastante utilizado, direta ou indiretamente, no tratamento dos pacientes com PH dos grupos I e IV(28).

Assim como acontece nos vasos pulmonares, estudos mostram que pode haver também disfunção endotelial periférica na PH e que essa disfunção parece estar correlacionada com a PVR e severidade da doença (29-31). Pacientes com PAH exibem pior vasorreatividade dependente do endotélio avaliada por fluxo na artéria braquialquando comparados com pacientes saudáveis (29). Dessa forma, evidências mostram que, apesar de a lesão inicial envolver os vasos pulmonares, a disfunção endotelial periférica também tem sido observada, sugerindo que pode haver vasculopatia generalizada na PH.

A análise da FMD durante a hiperemia reativa é o método não-invasivo mais utilizado para análise da função endotelial sistêmica e utiliza a ultrassonografia do vaso como ferramenta de avaliação. É uma medida dependente da vasodilatação mediada pelo NO na artéria braquial em resposta a tensão de cisalhamento de restrição temporária do fluxo sanguíneo e reperfusão (26). Dessa forma, uma melhor compreensão do funcionamento das células endoteliais e das respostas a diferentes estímulos, pode auxiliar não somente no melhor entendimento das doenças, mas também na busca por novos tratamentos auxiliares. A diminuição da biodisponibilidade do NO é ponto central no mecanismo,

provavelmente tanto no inicio quanto na manutenção da PH, justificando todo o investimento em tratamentos que aumentem a sua disponibilidade.

1.4 Balanço Simpatovagal

Como em outros órgãos, a interação dos sistemas nervosos simpático e parassimpático (SPNS) é importante para manter a homeostase dos sistemas cardiopulmonar e cardiocirculatório (32). No contexto das doenças cardiovasculares, normalmente há desequilíbrio entre esses dois sistemas:a simpatoexcitação costumacausar taquiarritmias e fiblilações cardíacas e vasoconstrição pulmonar e periférica, enquanto a ativação parassimpática costuma ser protetora para o coração e ser responsável pela vasodilatação (33, 34). No entanto, estudos em animais indicam que o sistema nervoso simpático também pode causar resposta pulmonar vasodilatadora, e de certa forma compensatória, através da ligação aos β receptores vasculares em situações de hipóxia (35) ou de tônus vasoconstritor pré-existente (34, 36).

O desbalanço na modulação do SPNS tem sido relatado como importante fator de risco para diversas doenças cardiovasculares (37-40)e morte súbita(37-40) e diversos estudos também já mostraram que existe alteração no SPNS em pacientes com PH (11, 41-43).

Velez-Roa e colaboradores, ao estudar a atividade simpática através de microneurografia em pacientes com PAH, encontraram aumento da atuação simpática e que este aumento pode estar relacionado com a severidade da doença(43).Dimopoulos e colaboradores também avaliaram a atividade do SPNS na PAH e encontraram profundas anormalidades na atuação desse sistema e que essas anormalidades relacionavam-se com

piora da capacidade de exercício e pior prognóstico(11). Ainda, Ciarka e colaboradores, em estudos sobre o mesmo sistema, concluíram que a ativação simpática é um preditor independente de deterioração clínica da PAH (43).

Estes resultados enfatizam a necessidade de estabelecer os possíveis mecanismos envolvidos nesta doença e, no futuro, contribuir para melhorar a qualidade de vida desses pacientes. Considerando que existe um desbalançosimpatovagal em muitas situações patológicas, inclusive na PH, a análise do controle deste sistema nervoso pode fornecer informações importantes de caráter diagnostico, mas principalmente de caráter prognóstico, podendo indicar o grau de comprometimento do sistema de controle cardiovascular.

A modulação simpatovagal para o coração pode ser avaliada a partir de um simples eletrocardiograma (ECG),que constitui um método não invasivo e de baixo custo de diagnóstico. A análise espectral, também conhecida como análise no domínio da frequência, é uma metodologia de grande aplicação clínica e potencialidade, a qual fornece uma avaliação quantitativa da modulação do SPNS e variabilidade da frequência cardíaca (HRV)sobre a função cardiovascular (44, 45). A HRV é a variação do período entre os batimentos cardíacos consecutivos em um determinado tempo (45). Quanto maior a ativação simpática, geralmente acompanhada de menor a HRV, maiores serão os riscos de doenças cardíacas (46).

A análise espectral de séries temporais de frequência cardíaca (HR) permite a avaliação da HRV e também a decomposição da variação da HR em componentes oscilatórios específicos, sendo eles definidos por sua frequência e amplitude. As oscilações rítmicas de HR apresentam três faixas distintas: a faixa HF (High Frequency), modulada pelo sistema nervoso parassimpático cardíaco; a LF (Low Frequency), relacionada à modulação simpática cardíaca; e a VLF (Very Low Frequency). (44, 45).

As medidas de função autonômica em pacientes com PAH podem ser clinicamente relevantes, uma vez que uma melhoria na modulação desse sistema poderia potencialmente reduzir os fatores de risco para eventos adversos cardiovasculares(47). A partir deste método barato e de fácil aplicabilidade, muitas intervenções, tanto farmacológicas quanto físicas, podem ser rapidamente avaliadas quanto ao benefício e resposta do paciente. Neste contexto, a avaliação quantitativa da modulação simpatovagal sobre o sistema cardiovascular pode auxiliar na estratificação de risco, monitoramento e quem sabe na pesquisa de possíveis tratamentos auxiliares para a PH.

1.5 Reabilitação Pulmonar

Os pacientes com PH são muitas vezes intolerantes ao exercício, apresentando acentuada dispnéia, fadiga ou fraqueza, que limitam a sua atividade de vida diária por causa de graves distúrbios cardiopulmonares(11). De fato, a redução no débito cardíaco contribui para uma diminuição da oferta de oxigênio aos tecidos, entre eles a musculatura periférica, e acentuam os sintomas descritos (2, 48), podendo contribuir para a diminuição da capacidade de praticar exercício.

Além disso, de acordo com recentes estudos, pacientes com PH também apresentam disfunção da musculatura respiratória e esta fraqueza inspiratória pode contribuir para a exacerbação dos sintomas da doença (49, 50). Somente em 2005, Meyer e colaboradores evidenciaram pela primeira vez que existe redução da força muscular inspiratória e expiratória dos pacientes com PAH idiopática e que esta fraqueza ocorre independentemente de perfil hemodinâmico, capacidade de exercício ou ineficiência ventilatória(49).

Os avanços na terapia farmacológica para a PAH têm apresentado resultados encorajadores no que se refere à capacidade de praticar exercício e ao perfil hemodinâmico. Contudo, a medicação não tem sido efetiva para combater totalmente a dispneia e a fadiga que continuam limitando a capacidade de realizar as atividades de rotina da vida diária. Essas dificuldades acabam levando a uma inatividade física com prejuízo à qualidade de vida desses pacientes (51) uma vez que acarreta um descondicionamento físico progressivo que culmina num quadro de limitação funcional (52).

Conforme o consenso do 5° Simpósio sobre Hipertensão Pulmonar realizado em 2013 em Nice, a reabilitação pulmonar e o treinamento físico supervisionados possuem nível de evidência I-A para o tratamento dos pacientes com PH (53). Este consenso foi definido de acordo com resultados de ensaios clínicos que avaliaram a reabilitação pulmonar nos pacientes com PH . De fato, Grünig e colaboradores avaliaram pacientes com PH antes e após 3 semanas de exercício físico e respiratório e concluíram que o exercício, utilizado como complemento ao tratamento farmacológico, pode melhorar a capacidade de exercício ea qualidade de vida nos pacientes com PH e que ele apresenta segurança em longo prazo(54). Ainda, Saglam e colaboradores avaliaram o treinamento da musculatura inspiratória (IMT) na PAH e concluíram que este exercício melhora significativamente a força muscular respiratória, capacidade funcional e percepção de dispneia e fadiga. Além disso, o programa de treinamento mostrou ser bem tolerado pelos pacientes e apresentou ótima aderência por parte deles, indicando que o IMT pode ser uma importante opção terapêutica (55).

A recomendação da fisioterapia, entretanto, apresenta algumas limitações devido ao conhecimento restrito com relação ao melhor método, intensidade e duração do treinamento. Além disso, os mecanismos associados à melhora dos sintomas, capacidade

funcional e possíveis efeitos sobre o prognóstico ainda não estão completamente entendidos (53), justificando nosso objetivo de identificar se esses pacientes, realizando o protocolo, também apresentariam melhora da capacidade funcional, da função endotelial e do balanço simpatovagal, com consequente redução do risco cardiopulmonar.

2OBJETIVOS

1. Demonstrar o balanço autonômico e a função endotelial periférica dos pacientes com hipertensão pulmonar;
2. Identificar se existe correlação entre a magnitude do desbalançosimpatovagal, a disfunção endotelial e a capacidade funcional nos pacientes com hipertensão pulmonar;
3. Verificar a resposta da modulação simpática e parassimpática e da função endotelial ao treinamento muscular inspiratório nos pacientes com hipertensão pulmonar.

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4 ARTIGOS CIENTÍFICOS

4.1 Artigo 1

Parasympathetic modulation withdrawal improves functional capacity in pulmonary arterial hypertension

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Original article

Parasympathetic modulation withdrawal improves functional capacity in pulmonary arterial hypertension

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Parasympathetic modulation withdrawal improves functional capacity Pulmonary Arterial Hypertension

Abstract

In 15 pulmonary arterial hypertension patients, the relation of functional capacity to their peripheral endothelial function and sympathaovagal modulation was studied by carrying out brachial artery ultrasound and electrocardiogram spectral analysis, respectively. The functional capacity was assessed by cardiopulmonary exercise testing and six-minute walking test. The sympathovagal modulation was correlated with the predicted peak oxygen consumption (peak VO_2 %; r=0.692, P<0.05), peak O_2 pulse (mL/beat; r=0.661, P<0.05), VE, minute ventilation, VCO_2 carbon dioxide production (VE/VCO_2 slope; r=-0.806, P<0.01) and distance walked predicted (%6MWT; r=0.694, P<0.05). Moreover, there were negative correlations between parasympathetic modulation with peak VO_2 (r=0.755, P<0.01), peak VO_2% (r=-0.727, P<0.01) and peak O_2 pulse (r=0.615, P<0.05), %6MWT (r=-0.834, P<0.01). Collectively these correlations indicate that parasympathetic withdrawal is crucial for improving functional capacity. This conclusion is supported by both positive and negative correlations of parasympathetic modulation with the functional capacity parameters. The sympathetic modulation predominance, although increases the cardiovascular risk, is probably crucial to facilitate the bronchodilation and the oxygen uptake.

Keywords:

Parasympathetic Nervous System

Sympathetic Nervous System

Functional Capacity

Pulmonary Arterial Hypertension

1. Introduction

Pulmonary arterial hypertension (PAH), which is characterized by endothelial cell dysfunction and progressive remodeling of the pulmonary vasculature (Galiè et al., 2009; Hoeper et al., 2006), leads to symptoms such as syncope, dyspnea and excessive fatigue that impair the functional capacity (Humbert, 2010; Sandoval et al., 1994). Studies have also suggested that there is an association between autonomic nervous system (ANS) imbalance and the severity of PAH (P. et al., 2015; Wensel et al., 2009). The sympathetic predominance tends to increase the heart rate (HR) and decrease the heart rate variability (HRV), increasing the risk of cardiovascular events (Dos Reis et al., 1998).

The sympathetic and parasympathetic pathways regulate not only the cardiovascular system, but also the internal organs. Thus, their modulation is crucial for monitoring risk stratification and, perhaps, might also be used to monitor the patient's clinical evolution. The quantitative assessment of sympathovagal modulation can be assessed by an electrocardiogram (ECG), a noninvasive method that substantially facilitates its use in clinical practice to perform spectral analysis (Berntson et al., 1997; Campos et al., 2013).

Furthermore, a great predictor of poor prognosis in PAH is the ventilatory response to exercise (Wensel et al., 2002), whereas the principal clinical manifestations of right heart failure are exercise limitation and edema (Benza et al., 2010). For PAH patients, a six-minute walking test (6MWT) can be used as a submaximal evaluation, while the incremental symptom-limited cardiopulmonary exercise testing (CPET) is usually performed as a maximal exercise test. Collectively, both tests provide important information about functional capacity, as well as gas exchange, ventilatory efficacy and cardiac function even during light exercise (Miyamoto et al., 2000; Sun et al., 2001).

Recent studies have also shown that both systemic and pulmonary vascular endothelium dysfunction might exist in PAH patients (Peled et al., 2009, 2008). This is manifested by excessive pulmonary vascular cell proliferation, smooth muscle cell apoptosis inhibition, endogenous vasoconstrictors and vasodilators imbalance, and a decrease in nitric oxide (NO) bioavailability (Friedman et al., 2012). Although the initial injury in PAH involves the pulmonary vasculature, systemic endothelial dysfunction has also been observed (Gabrielli et al., 2011; Peled et al., 2009).

Considering that patients with PAH have: 1) increased carotid chemoreceptor activity (Velez-Roa et al., 2004) and sensitivity (Farina et al., 2018), and 2) poor post-exercise cardiac autonomic control (Da Silva Gonçalves Bós et al., 2018; Minai et al., 2012; Ramos et al., 2012), and that the clinical information provided by CPET and 6MWT in PAH is important to predict survival (Grünig et al., 2013), our aim was to investigate whether there was an association among functional capacity parameters with the peripheral endothelial function and the sympathovagal modulation in these patients.

Thus, demonstrating an association between the sympathovagal modulation and other parameters provides important knowledge about the pathophysiology of the disease and, consequently, its management. Evaluation of the ANS can be carried out easily and safely in the physician's office through a simple ECG. This information could improve monitoring of the patient's clinical evolution and make intervention possible, allowing adjustment of the sympathovagal modulation, to simultaneously improve breathing and decrease cardiovascular risk in PAH patients.

2. Methods

The study protocol was approved by the local research ethics committee and was performed in accordance with the Declaration of Helsinki. Informed written consent was obtained from all participants. This study was registered as ‘‘Study of the effect of inspiratory muscle training on endothelial function, autonomic control, exercise capacity of life in patients with pulmonary hypertension’’ and was conducted at a single center specializing in respiratory care on the Brazilian protocol registry (Brazilian protocol registry: RBR-33gm3k- http://www.ensaiosclinicos.gov.br/rg/RBR-33gm3k/).

2.1 Study population

Fifteen patients taking vasodilator therapy were invited to participate. The patient's diagnosis was performed according to pulmonary hypertension guidelines (Galiè et al., 2016), and they were screened through patient chart, according to inclusion and exclusion criteria. Pulmonary hypertension was confirmed by right heart catheterization (RHC), and all patients were in group 1 of pulmonary hypertension – PAH. Thus, all patients had similar clinical presentation, pathological findings, hemodynamics characteristics and treatment strategy (Galiè et al., 2016).

2.2 Inclusion criteria

PAH patients ≥18 years old belonging to group 1 of pulmonary hypertension and functional class II or III (World Health Organization) were included in the study. Patients were required to be stable for at least the last three months prior to the start of the study.

2.3 Exclusion criteria

Patients were excluded when they presented: musculoskeletal disorders, intermittent pain, cognitive or neurological deterioration, history of moderate or severe chronic lung disease, hemodynamic instability, unstable angina, uncontrolled cardiac arrhythmia, or psychiatric-psychological disorders that may interfere in their understanding of the protocol. Subjects who were hospitalized in the last three months and/or were using oxygen and those participating in supervised exercise programs in the past three months were also excluded from the study.

2.4 Protocol

This is an observational study to evaluate the association among functional capacity parameters with the peripheral endothelial function and the sympathovagal modulation of PAH patients.

After the screening, patients started the protocol tests. During the first visit, patients underwent clinical evaluation, pulmonary function testing, and CPET. Four days later (on the second visit), the ECG, peripheral endothelial function test and 6MWT were performed. Previous examinations, such as RHC, vital signs, and clinical information collected in the hospital database, were also considered.

2.5 Maximal exercise capacity (incremental exercise test)

The CPET was performed according to the standardization of the American Thoracic Society (ATS)/American College of Chest Physicians to assess the maximal exercise capacity (Arena et al., 2010). All exercise tests were performed on an electromagnetically braked cycle ergometer (Corival®; Lode, Groningen, the Netherlands), with the use of a computer-based breath-by-breath CPET system (Vmax29®-SensorMedics). HR was determined from the R-R interval of a 12-lead ECG, and oxygen saturation was measured by pulse oximetry. All CPET variables were presented as 20-s averages. During incremental CPET, the workload was increased every 1 min from a baseline of 2 min of load less pedaling at a rate of 5-10 W/min to maximum tolerance, and gas exchange measurements were recorded until maximal exertion. The protocol consisted of 3 minutes at rest on the bicycle, followed by 2 minutes of cycling without load (0 W); subsequently the workload was increased by 5 W/min or 10 W/min depending on age, gender, body mass and functional exercise capacity. The dyspnea and fatigue data were recorded every 2 minutes and evaluated with the modified CR10 Borg Scale (0-10) (Borg, 1982). The following ventilatory parameters were acquired: minute ventilation (VE), O_2 uptake (VO_2), peak VO_2 and CO_2 output (VCO_2). The anaerobic threshold was determined by the V-slope method. The VE/VCO_2 slope was calculated during exercise with all data range. These parameters reflect the ventilatory efficiency and oxygen consumption, which are considered prognostic indicators for patients with heart failure (Arena et al., 2010; Galiè et al., 2016).

2.6 Electrocardiogram

All ECGs were recorded (10 minutes) in the morning on fasted patients. During data recording, the subjects were at rest in the supine position in a quiet room. They were

advised to remain motionless in a comfortable position. Disposable electrodes were placed on the skin of the chest to collect electrocardiographic signals (derived DI, DII and DIII,) which reflect the cardiac electrical activity (Guimarães et al., 2003). Continuous ECG signals (sampling rate, 600 Hz) were recorded by a Wincardio system (MicromedBiotecnologia – bath 53, series nº 1444107, ANVISA registration 10307270007) and used to perform spectral analysis using an autoregressive model. All female patients performed the ECG while they were on days 1 to 5 of the menstrual cycle.

2.7 Sympathetic and parasympathetic modulation evaluation

The sympathetic and parasympathetic modulation of the heart was evaluated by spectral analysis of a time series of R-R intervals (tachograms) extracted from the ECG signals. After detecting the pulse intervals (PIs), the heart period was automatically calculated on a beat-to-beat basis as the time interval between two consecutive systolic peaks or a PI. All data were carefully checked to avoid erroneous detections or missed beats. Sequences of 200-300 beats were randomly chosen. If the randomly selected sequence included evident non-stationarities, the sequence was discarded, and a new random selection was performed. Frequency domain analysis of HRV was performed with an autoregressive algorithm (Dias Da Silva et al., 2002; Malliani et al., 1991; Porta et al., 2001) on the PI interval sequences (tachogram). The power spectral density was calculated for each time series. In this study, two spectral components were considered: low frequency (LF), from 0.04 to 0.15 Hz, and high frequency (HF), from 0.15 to 0.5 Hz. The spectral components are expressed in absolute (a; ms^2) and normalized units (nu; %). Normalization consisted of dividing the power of a given spectral component by the total power and multiplying the ratio by 100 (Montano et al., 1994). Moreover, the ratio of the

absolute LF/HF values, known as cardiac sympathetic and parasympathetic balance, was also calculated for each stretch (Montano et al., 2009).

2.8 Endothelial function

All endothelial tests were performed in the morning during the second visit; patients were fasted and without medication. Endothelial function was noninvasively assessed with an ultrasound probe (EnVisor Series, Philips Ultrasound, Bothell, WA) and doppler ultrasonography using an instrument equipped with a 7- to 12-MHz high-resolution linear probe (L12-3, Philips, Bothell, WA, USA). This exam was performed according to the guideline, in a temperature-controlled and silent room. The left brachial artery diameter was measured from B-mode ultrasound images at these conditions: at rest, during reactive hyperemia and after nitroglycerin (NTG) administration. A resting scan was performed before blood pressure (BP) cuff inflation (50mm Hg above systolic BP). When the cuff was inflated, it was placed the forearm and the arterial blood occluded for 5 minutes. This procedure causes ischemia and, consequently, a vasodilation via auto regulatory mechanisms. A second continuous scan was recorded from 30–120 seconds after the cuff deflation. Another scan was acquired after 10 minutes of rest to reflect the reestablished subject baseline conditions. An NTG spray was then administrated by sublingual mode and, after some minutes (3-4), the fourth scan was recorded, in order to measure the endothelium-independent vasodilation. The same experienced sonographer performed all of the ultrasound scans but had no information about the subjects. The vessel diameter was measured after the examination, in offline mode, at a fixed position with ultrasonic calipers at end-diastole, and incident with the R wave on a continuously recorded ECG. The dilatation was obtained using the difference from baseline and after

10-second intervals during the period from 30-180 seconds. The flow-mediated dilatation (FMD) value (%) indicates the blood flow increase after release of the sphygmomanometer cuff. The endothelium-independent vasodilation value (%) indicates the maximum vasodilator response from the exogenous NO donor (Corretti et al., 2002).

2.9 The six-minute walking test

The 6MWT was conducted for all patients according to a standardized protocol as described in the ATS guideline ("ATS Statement," 2002). The subjects walked for 6 minutes along an enclosed 30-m long corridor in the hospital and were instructed to walk at their own pace to cover as much distance as possible. The HR (Polar heart rate monitor, PolarS810i; Polar Electro, Finland), was monitored and oxygen saturation (Risingmed, RMS-50D, Beijing, China) was measured during the test. Dyspnea and fatigue perception were determined using the modified CR10 Borg Scale (0-10) before and after the 6MWT (Borg, 1982). All PAH patients had previously performed the 6MWT, thus, the test was performed only once (Holland et al., 2014). We calculated the walking distance (D6MWT) and a Brazilian study was used to calculate the predicted values of normality (%6MWT) (Britto et al., 2013). Body mass was used to calculate the 6-min walk work (6MWw) [distance (m) x body weight (kg)]. Measurements were made with a calibrated Filizola scale (0.1 kg of precision) and with a stadiometer (0.5 cm of precision) to calculate the body mass index (BMI).

2.10 Statistical analyses

The Shapiro-Wilk test was performed to assess the data distribution. Spearman's correlation test was used to assess the linear association between continuous variables.

Data are expressed as frequency and percentage for categorical variables and as mean±SD for continuous variables. Statistical significance was accepted at $P \leq 0.05$. All results were analyzed using commercial software (SPSS ver. 20, SPSS Inc., Chicago, IL, USA; Excel 2010, Microsoft Corporation, Redmond, WA, USA).

3. Results

Table 1 summarizes the patients' clinical characteristics, RHC, pulmonary function test, CPET, and 6MWT results. All patients were of female gender, their average age was 40.1± 6.7 years old and WHO functional class II or III.

3.1 Endothelial function

Collectively, our data demonstrated that the basal brachial artery dilates 3.8±3.4% (FMD). Although expected for PAH patients, the FMD is reduced. This result indicates that there is not enoughNO to induce the correct dilatation. On the other hand, the NTG response showed a normal dilatation (21±8%). Although endogenous NO is not enough, this result indicates that the brachial artery is still able to dilate with the administration of a NO donor (NTG). Despite that, we did not find any association between the endothelial function and the parameters measured in this study.

3.2 Functional capacity and ANS modulation

Table 2 shows the spectral analysis results. Our data demonstrated a predominance of the sympathetic modulation over the parasympathetic nervous system, seen by the LF/HF ratio. Moreover, there was a negative correlation between the parasympathetic modulation in absolute values (HFa – ms^2) and the following functional capacity

parameters: peak VO_2, peak VO_2%, peak O_2 pulse, D6MWT, %6MWT, and 6MWw (Table 3). There was also a negative correlation between HRV and the parameters of peak O_2 pulse, peak VO_2%, 6MWw and %6MWT, and also between the LF/HF ratio and the VE/VCO_2 slope (Table 3 and Figure 1- D).

On the other hand, there was a positive correlation between the LF/HF ratio and the parameters of peak VO_2, peak VO_2 %, peak O_2 pulse and %6MWT (Table 3 and Figure 1-A, B and C).

Overall, these correlations indicate that the greater the parasympathetic participation, the worse the patient functional capacity. This conclusion is supported by both positive and negative correlations, since the parasympathetic withdrawal, which proportionally increases the sympathetic participation, as seen by the higher LF/HF ratio, is associated with better functional capacity. Likewise, the HRV, which occurs when parasympathetic modulation decreases and proportionally increases sympathetic participation, is inversely associated with better functional capacity.

4. Discussion

This is the first study to demonstrate that sympathetic dominance in PAH patients is probably due to the parasympathetic withdrawal. This withdrawal is corelated with an improvement in functional capacity, emphasizing the importance of sympathetic participation for the respiratory system in these patients. The peak O_2 pulse, peak VO_2,VO_2 %, %6MWT, and 6MWw were inversely associated with HFa. On the other hand, due to the proportionally lower parasympathetic participation, the sympathovagal balance changed to the sympathetic domain, probably improving oxygen uptake.

It is very well established in the literature that the sympathetic modulation is dominant in PAH patients and this predominance is related to the severity of heart failure and, consequently, poor outcome (Ciarka et al., 2010; Velez-Roa et al., 2004). Furthermore, Ciarka et al., report that sympathetic activation was related not only to the progression of heart failure but also to exercise intolerance in a population of patients with advanced PAH (Ciarka et al., 2010). Therefore, there is no doubt that the dominance in sympathetic modulation is associated with an increase in cardiovascular risk. Conversely, regarding the respiratory pathophysiology, the benefits of decreasing sympathetic participation should be considered with caution.

Our results also demonstrated a negative correlation between the HRV and the peak VO_2. A possible explanation for this finding could be the withdrawal of parasympathetic modulation, which reduced the total power of HR in favor of sympathetic participation. The increase in sympathetic modulation facilitated the O_2 uptake. Furthermore, it is also known that the HR is unstable in patients with heart failure (Kleiger et al., 2005), which could also ultimately explain the inverse correlation found in PAH patients.

In addition, it is important to emphasize that our results do not express a sympathetic overactivation. They express only a sympathetic predominance due to parasympathetic withdrawal. In heart diseases, data from the literature show that the LFa band of the spectral components is from 393 to 598 ms^2, and the HFa band is from 198 to 240 ms^2 (Can et al., 2013; Fauchier et al., 2004), which agrees with our findings. We demonstrated that the bigger the parasympathetic withdrawal, the better the PAH patient is, from the respiratory point of view. This is also substantiated in the strong negative correlation observed between the parasympathetic modulation and the functional capacity found in our study. These rationale are based on the findings of Wensel et al (Wensel et

al., 2009), who while comparing PAH patients with healthy controls, observed a marked reduction in HF power indicating that there is a decrease in parasympathetic modulation in the former.

Thus, we concluded that the sympathetic predominance is probably favoring the functional capacity, inducing broncho and vasodilation in the pulmonary bed. This conclusion is consistent with Shirai et al. who demonstrated in cats that, during hypoxia, the sympathetic modulation plays an important role in facilitating vasodilation by β-adrenergic receptors (Shirai et al., 1995). According to these authors, this increased sympathetic modulation would prevent excessive pulmonary hypertension during systemic hypoxia.

In accordance with Shirai and co-workers, our results demonstrated that the patients' functional capacity is positively related to the compensatory withdrawal of parasympathetic participation (Shirai et al., 1995). At least at rest, this withdrawal probably favors sympathetic predominance and consequent homeostasis. Furthermore, considering the great complexity and diversity of autonomic participation, the proportion of the parasympathetic participation post-exercise is not guaranteed during exercise or at rest.

On the other hand, the patient´s pathophysiology does not change at rest compared to during exercise. The body's response to exercise is in accordance with the resting body condition. This is supported by the positive association between 6MWT results and the “long-term prognosis”, which reflects the patient’s organic status (Galiè et al., 2016; Souza et al., 2018). Moreover, exercise training modulates diverse molecular mechanisms that are associated with proliferation, apoptosis, oxidative stress, inflammation, proteolysis and vasodilatation (Nogueira-Ferreira et al., 2018) and which

are, no doubt, present at rest or during exercise. This evidence strengthens our rationale that the functional parameters evaluated in our study reflect more than a single moment.

There is also no doubt that the great impairment in the autonomic balance in these patients worsens the HRV. Further studies will be necessary to demonstrate the ideal proportion between the sympathetic and parasympathetic nervous system to provide welfare with lower cardiovascular risk, benefiting both the cardiocirculatory and respiratory systems.

Although the endothelial function did not show a significant correlation with any functional parameters, a possible reason could be that the patients were taking vasodilators that very likely masked the endothelial response to various stimuli. Our data 'agrees' with findings of others, in that there is a peripheral endothelial dysfunction in PAH patients (Peled et al., 2009; Wolff et al., 2007), which reinforces our hypothesis above.

Another point that we wish to highlight in relation to the findings of this study is that the pathophysiological compensation of the patients could be on the limit. The autonomic modulation is pushing the cardiopulmonary system to keep the tissue perfusion and the medication is contributing to the necessary vasodilation. Moreover, a limitation of our study is the patient's medication, which probably interfered with the pathophysiological responses, and also the heterogeneous PAH etiology of the participants.

On the other hand, we strongly believe that, despite the severity of the disease, the predominance of the sympathetic nervous system modulation, due to the fundamental parasympathetic withdrawal, might act in order to compensate the respiratory failure, improving the functional capacity in these PAH patients.

5. Conclusion

In conclusion, considering the bigger proportional decrease in HFa compared to LFa, and the consequent increase in LF/HF ratio, our findings demonstrate that the sympathetic participation dominance is probably due to a proportional reduction in the parasympathetic modulation. In addition, the LF/HF ratio at rest was correlated to the CPET, demonstrating the physiological significance of the sympathetic dominance to the respiratory system.

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7. Disclosures

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8. Declarations of interest: none.

8.1 Sample CRediT author statement:

Gabriela Roncato: Conceptualization, methodology, formal analysis, writing - original draft; **Fabrício Farias da Fontoura:** Conceptualization, methodology, formal analysis, investigation, writing - original draft; **Fernanda Brum Spilimbergo:** investigation, writing - review & editing; **Gisela Martina Bohns Meyer:** investigation, writing - review & editing; **Guilherme Watte:** Conceptualization, formal analysis, investigation, writing - original draft; **Walter Oliveira de Vargas:** investigation; **Karina Rabello Casali:** investigation, writing - review & editing; **Danilo Cortozi Berton:** Conceptualization, methodology, writing - review & editing, supervision; **Katya Rigatto:** Conceptualization, methodology, formal analysis, writing - original draft, writing - review & editing, supervision.

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10. Figure Legends

Figure 1.

Correlations between LF/HF ratio and cardiopulmonary exercise testing. A) Positive correlation between the LF/HF ratio and peak VO_2; B) Positive correlation between the LF/HF ratio and peak O_2 pulse; C) Positive correlation between the LF/HF ratio Peak VO_2 % predicted. D) Negative correlation between the LF/HF ratio and the VE/VCO_2 slope. VO_2, oxygen consumption; O_2, oxygen; VE, minute ventilation; VCO_2, carbon dioxide production; LF, low-frequency component; HF, high-frequency component; LF/HF, ratio between low- and high-frequency power components**.**

Figure 1

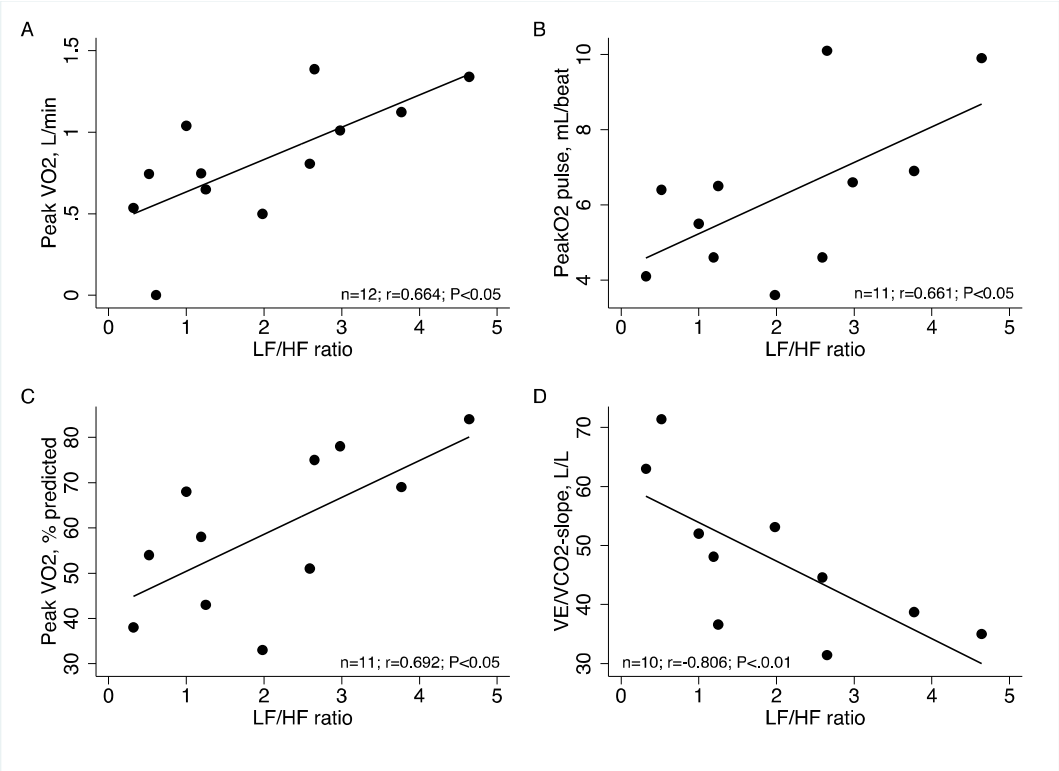
A
Peak VO2, L/min
0
.5
1
1.5
n=12; r=0.664; P<0.05
0
1
2
3
4
5
LF/HF ratio
B
PeakO2 pulse, mL/beat
4
6
8
10
n=11; r=0.661; P<0.05
0
1
2
3
4
5
LF/HF ratio
C
Peak VO2, % predicted
30
40
50
60
70
80
n=11; r=0.692; P<0.05
0
1
2
3
4
5
LF/HF ratio
D
VE/VCO2-slope, L/L
30
40
50
60
70
n=10; r=-0.806; P<.0.01
0
1
2
3
4
5
LF/HF ratio

Table 1. Demographic and clinical characteristics of the patients.

Characteristic	N=15
Female	15 (100)
Age (years)	40.1±6.7
BMI (kg/m^2)	26.8±5.7
SBP (mmHg)	108.0±11.8
DBP (mmHg)	77.7±9.9
WHO/NYHA	
Class II	11 (73.3)
Class III	4 (26.6)
PAH etiology	
Congenital heart diseases (corrected)	3 (20.0)
Congenital heart diseases (uncorrected)	3 (20.0)
Connective tissue disease	4 (26.6)
Idiopathic	3 (20.0)
HIV	2 (13.3)
Drug therapy	
ERA	7 (46.6)
ERA + PD5i	8 (53.3)
Pro-BNP (pg/mL)	514±569
Right heart catheterization	
PAPm (mmHg)	52.0±14.5
Cardiac output (L/min)	4.9±1.4
PVR (Wood)	9.1±4.6
Pulmonary function test	
FEV_1(%)	75.3±15.4
FVC (%)	81.1±12.8
FEV_1/FVC (%)	0.76±0.1
Peak cardiopulmonary exercise testing	
VO_2 (mL.kg^{-1}.min^{-1})	13.6±4.4
VO_2 (L/min)	0.88±0.3
VO_2 (% predicted)	57.6±16.3
VE/VCO_2-slope (L/L)	47.7±12.8
O_2 pulse (mL/beat)	6.2±2.0
SpO_2 (%)	92.6±9.5
SBP (mmHg)	138.8±23.8
RER (VCO_2/VO_2)	1,17±0,16
Borg dyspnea scores (0-10)	5.7±3.3
Borg fatigue scores (0-10)	7.1±2.5
Six-minute walking test	
Distance (m)	506.0±49.9
Distance (% predicted)	86.5±10.5
Peripheral O_2 desaturation (%)	6.1±9.3

Values are n (%) or mean ± standard deviation. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WHO, World Health Organization; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; HIV, Human Immunodeficiency Virus; ERA, endothelin receptor antagonist; PD5i, phosphodiesterase type 5 inhibitor; Pro-BNP, N-terminal prohormone of brain natriuretic peptide; PAPm, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; FEV_1, forced expiratory volume of 1 second; FVC, forced volume vital capacity; FEV_1/FVC ratio, Tiffeneau-Pinelli index; O_2, oxygen; VO_2, oxygen consumption;VE, minute ventilation; VCO_2, carbon dioxide production; m, meters; SpO_2, saturation of peripheral oxygen; RER, respiratory exchange ratio

Table 2. Spectral analysis (n=13)

Variables	Mean±SD
Heart Rate (bpm)	69.5±10
Heart Rate Variability (ms^2)	1409±1503
LF peak (Hz)	0.04±0.01
LF band (ms^2)	512±887
LF band (nu)	58±19
HF peak (Hz)	0.3±0.1
HF band (ms^2)	367±475
HF band (nu)	41±19
LF/HF ratio	1.9±1.3

SD, standard deviation; LF, low-frequency component; HF, high-frequency component; LF/HF, ratio between low- and high-frequency power components; bpm, beats per minute; nu, normalized units.

Table 3. Correlations between spectral analysis and cardiopulmonary exercise testing or six-minute walking test.

	HR (bpm)	HRV (ms^2)	LFa (ms^2)	HFa (ms^2)	LFnu (%)	HFnu (%)	LF/HF ratio
Cardiopulmonary exercise testing							
Peak VO_2, $mL.kg^{-1}.min^{-1}$ (n=12)	0.545	-0.189	-0.118	-0.436	0.418	-0.418	0.418
Peak VO_2, mL/min (n=12)	0.827†	-0.455	-0.373	-0.755†	0.664*	-0.664*	0.664*
Peak VO_2, % predicted (n=11)	0.748†	-0.626*	-0.350	-0.727†	0.692*	-0.692*	0.692*
Peak O_2 pulse, mL/beat 9n=11)	0.661*	-0.611*	-0.314	-0.615*	0.661*	-0.661*	0.661*
VE/VCO_2-slope (n=10)	-0.515	0.409	0.139	0.515	-0.806†	0.806†	-0.806†
Six-minute walking test (n=13)							
Distance, m	0.615*	-0.462	-0.273	-0.580*	0.476	-0.476	0.476
Distance, % predicted	0.830†	-0.766†	-0.567	-0.834†	0.694*	-0.694*	0.694*
6MWw, kg/m	0.755†	-0.797†	-0.627	-0.800†	0.509	-0.509	0.509
Dyspnea Borg	-0.402	0.109	0.316	0.514	-0.359	0.359	-0.359
Fatigue Borg	-0.464	0.448	0.547	0.611*	-0.286	0.286	-0.286

HR, heart rate; HRV, heart rate variability; LF, low-frequency component; HF, high-frequency component; LF/HF, ratio between low- and high-frequency power components; bpm, beats per minute; nu, normalized units; VO_2, oxygen consumption; VE, minute ventilation; VCO_2, carbon dioxide production; 6MWw, 6-min walk work = dist□□e (m) x body weight (kg); P < 0.05 w□s □o□sidered st□tisti□□lly sig□ifi□□t. *P<0.05; †P<0.01

4.2 Artigo 2

Does Inspiratory Muscle Training improve the Sympathovagal Balance and Endothelial Function in Groups I and IV of Pulmonary Hypertension?

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Artigo formatação *CirculationJournal* – Fator de impacto: 4,124

May 19st, 2017

To: The Editors
Circulation Journal

Please find enclosed the manuscript “Does Inspiratory Muscle Training improve the Sympathovagal Balance and Endothelial Function in Groups I and IV of Pulmonary Hypertension?” for consideration of publication in the American Heart Journal.

The manuscript complies with the journal author guideline and the present work with international research ethical standards. All authors declare that they have no conflicts of interest and will not submit this work elsewhere while under consideration for publication.

Warmregards,

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Does Inspiratory Muscle Training improve the Sympathovagal Balance and Endothelial Function in Groups I and IV of Pulmonary Hypertension?

Inspiratory Training in Pulmonary Hypertension

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Does Inspiratory Muscle Training improve the Sympathovagal Balance and Endothelial Function in Groups I and IV of Pulmonary Hypertension?

Abstract

Introduction: Pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension are two subgroups of Pulmonary Hypertension (PH). The pharmacological treatment is usually with vasodilators, but it could be complemented by pulmonary rehabilitation. Thus, considering that PH patients usually have respiratory weakness, the inspiratory muscle training (IMT) could improve treatment to this disease. Our aim was to investigate the effect of IMT on peripheral endothelial function and sympathovagal balance in PH patients. Methods and results: Twelve patients in functional class II performed IMT using an inspiratory (resistor linear) threshold-loading device (Power Breathe® Plus Medium) in two sets of thirty large respiratory movements-diaphragmatic breathing, twice a day for 8 weeks. The patients performed respiratory muscle strength evaluation, six-minute walking test, electrocardiogram and a brachial doppler ultrasonography before and after the treatment. The spectral analysis showed significant decrease in the LF component, or sympathetic modulation in normalized units (LFnu baseline=59±21 and after IMT=49±17; p=0.048) and increase in the HF component, or parasympathetic modulation (HFnu baseline=41±21 and after IMT=51±17; p=0.048). Also, the sympathovagal balance, seen by the LF/HF ratio, decreased significantly after IMT (LF/HF ratio baseline=2.0±1.4 and after IMT=1.2±0.8; p=0.030). Conclusion: Although the peripheral endothelial function did not show significant difference, the IMT

improved the sympathovagal balance in these PH patients, probably reducing the cardiovascular risk.

Keywords

PulmonaryArterial Hypertension

Parasympathetic Nervous System Modulation

Sympathetic Nervous System Modulation

Inspiratory Muscle Training

Introduction

Pulmonary Hypertension (PH) is a pathophysiological disorder with multiple clinical conditions characterized by vascular dysfunction that restricts the pulmonary circulation and compromises the quality of life of patients. It is classified in five groups, according to pathological characteristics, hemodynamic findings, clinical presentation and treatment. Pulmonary arterial hypertension (PAH - Group I) and chronic thromboembolic pulmonary hypertension (CTEPH – Group 2) are two examples of PH (1).

The pulmonary vasculature damage occurs through cellular proliferation, tissue remodeling, vasoconstriction, inflammation and thrombosis, leading to a gradual increase pulmonary vascular resistance (PVR) (2, 3). The high PVR leads to an increase in right ventricle (RV) post-load, compensatory right ventricular hypertrophy and, frequently,

right heart failure (RHF) (4, 5). Although the initial injury occurs in the pulmonary vessels, studies show that there is also peripheral endothelial dysfunction and the dysfunction appears to be correlated with PVR and the disease severity (6-8).

The cardiac output decrease, caused by RHF, reduces the oxygen supply to the skeletal muscles that leads to fatigue, dyspnea and exercise intolerance (9, 10). In addition, PH patients often have respiratory muscular weakness, which contributes to the exacerbation of symptoms (11, 12). The pharmacological treatment is usually with vasodilators drugs and could be complemented by pulmonary rehabilitation, such as inspiratory muscle training (IMT) (1).

Moreover, the sympathetic and parasympathetic imbalance is also present many cardiovascular diseases (13-15), including PH (16, 17). Studies showed that the sympathetic nervous system modulation is increased in PH and it may be correlated with disease severity (16, 17). Thus, the sympathovagal modulation evaluation, by spectral analysis, could bring important information about risk stratification, monitoring and response to treatments.

Therefore, the aim of this clinical prospective study was to investigate the effect of IMT on peripheral endothelial function and sympathovagal balance in PH patients.

Materials and Methods

The local ethics research committee approved this study and it was performed in accordance with the Declaration of Helsinki. All subjects consented and signed the Informed consent. This study was registered as ‘‘Study of the effect of inspiratory muscle training on endothelial function, autonomic control, exercise capacity of life in patients

with pulmonary hypertension'', conducted at a single center specializing in respiratory care (Brazilian protocol registry number RBR-33gm3k - http://www.ensaiosclinicos.gov.br/rg/RBR-33gm3k/).

Study population

Patients with PAH or CTEPH followed at Santa Casa de Misericordia de Porto Alegre were screened according to inclusion and exclusion criteria. The PH diagnosis was performed in accordance to the pulmonary hypertension guideline (1). Eighteen subjects were including in this study, but six of them didn't complete the protocol due to withdrawal.

Inclusion criteria

Patients with pulmonary hypertension confirmed by right heart catheterization (RHC)with 18 years or morebelonging to group I or IV of pulmonary hypertension with inspiratory muscule weakness. They must be in functional class II or III of World Health Organization and with specific drug treatment for pulmonary hypertension stable for at least three months.

Exclusion criteria

Patients with significant musculoskeletal disorders, intermittent pain, cognitive or neurological deterioration, history of moderate or severe chronic lung disease, hemodynamic instability, unstable angina or uncontrolled cardiac arrhythmia, or with psychiatric-psychological disorders that may interfere in the understanding of the protocol. Subjects who were hospitalized in the last three months, in use of oxygen and who participated in supervised exercise programs in the past three months.

Protocol

Women with clinically stable PAH or CTEPH participated to this protocol. These patients performed IMT using an inspiratory (resistor linear) threshold-loading device (Power Breathe® Plus Medium) in two sets of thirty large respiratory movements-diaphragmatic breathing (18), twice a day for 8 weeks. Nine patients received IMT at 50% of maximal inspiratory pressure (MIP) and three IMT without load. The pressure was measured once a week in the hospital to adjust the resistance in order to maintain MIP. Before and after the protocol, respiratory muscle strength, six-minute walking test, electrocardiogram (ECG) and a brachial doppler ultrasonography were performed by the patients.

After screening, the patients started the baseline evaluation. At the baseline visit, patients underwent clinical evaluation and respiratory muscle strength test. At second visit, after 4 days, the patients underwent ECG, brachial doppler ultrasonography, pulmonary function test and six-minute walking test (6MWT), respectively. In addition,

the data were compiled in a database with results from previous exams, as RHC, vital signs and clinical information.

Respiratory muscle strength evaluation

The MIP and maximal expiratory pressure (MEP) were assessed using a calibrated digital mouth pressure meter (Globalmed, MVD 300®) with instructions and a demonstration being given prior to the tests. All measurements were performed using the nose clip and with a 2-mm wide opening in the mouthpiece to prevent overestimation of values because of glottal closure and pressure by the mouth muscles (19). The MIP was measured near a residual lung volume after a maximal expiration and the MEP was measured near total lung capacity after a maximal inspiration and both were conducted in an upright-seated position with legs and trunks supported (19). To measure MEP, the researcher pressed the cheeks of the volunteers to prevent air leakage (19). A minimum of five maneuvers at 1-minute intervals were conducted, with the variability between the best three measurements less than 10% and the other with a variation of no more than 20% with pressure of higher value. The minimum operating time was 1.5 seconds so that the maximum sustained pressure could be observed for 1 second, MIP and MEP average were determined by calculating the shaded area (19). The largest measure could not be the last test because of a potential learning effect and the highest pressure value was selected.

Pulmonary function test

Spirometric tests were performed with a calibrated pneumotachograph (Vmax29®; SensorMedics, Yorba Linda, CA, USA) and were performed in accordance with international standards (20). The variables obtained were expressed as absolute and percent predicted values (21).

Electrocardiogram

All ECGs were recorded during 10 minutes on patients fasted in the morning. During the data recording, the subjects were advised to remain motionless in a comfortable supine position, in a quiet room. Electrocardiographic signals were collected (derived DI, DII and DIII) to cardiac electrical activity monitoring (22). Continuous ECG signals (sampling rate, 600 Hz) were recorded by a Wincardio system (MicromedBiotecnologia – bath 53, series nº 1444107, ANVISA registration 10307270007) and used to perform spectral analysis using an autoregressive model. All female patients performed the ECG while they were on days 1 to 5 of the menstrual cycle.

Sympathetic and parasympathetic modulation evaluation

The sympathovagal modulation of the heart was evaluated by spectral analysis of a time series of RR intervals (tachograms) extracted from the ECG signals through

software provided by the manufacturer of the acquisition system (Acknowledge software, Biopac Systems Inc).

The pulse intervals were detecting and, after, the heart period was automatically calculated on a beat-to-beat basis as the time interval between two consecutive systolic peaks or a pulse interval. All data were checked to avoid missed beats or erroneous detections and sequences of 300 beats were randomly chosen. If the randomly selected sequence included evident non-stationarities, the sequence was discarded, and a new random selection was performed. An autoregressive algorithm was performed to frequency domain analysis of heart rate variability (HRV) (23-25) on the pulse intervals sequences (tachogram). In the present study, we considered two spectral components: low frequency (LF), from 0.04 to 0.15 Hz, and high frequency (HF), from 0.15 to 0.5 Hz. The spectral components are expressed in absolute (s2 or mmHg2) and normalized units (nu). Normalization is the division of the power of a given spectral component by the total power and multiplying the ratio by 100 (26). The ratio of the absolute LF/HF values, known as cardiac sympathovagal balance, was also calculated for each stretch (27).

Endothelial function

All endothelial tests were performed on patients fasted in the morning, without medication consumption. Also, as recommended in the guideline, the exams were performed in a temperature-controlled and silent room. Noninvasively endothelial function was assessed by means of a brachial artery ultrasound probe (EnVisor Series, Philips Ultrasound, Bothell, WA) and doppler ultrasonography by instrument equipped with a 7- to 12-MHz high-resolution linear probe (L12-3, Philips, Bothell, WA, USA).

The left brachial artery diameter was measured from B-mode ultrasound images at these conditions: at rest and during reactive hyperemia. A resting scan was performed before blood pressure (BP) cuff inflation (50 mmHg above systolic BP). When the cuff was inflated, it was placed around the forearm, occluded arterial for 5 minutes. This procedure causes ischemia and, consequently, a vasodilation via auto regulatory mechanisms. A second continuous scan was recorded from 30–120 seconds after the cuff deflation. The same experienced sonographer performed all of the ultrasound scans, and he had no information about the subjects. The vessel diameter was measured after the exam, in mode offline, at a fixed position with ultrasonic calipers at end-diastole, and incident with the R wave on a continuously recorded electrocardiogram. The dilatation was obtained by the difference from baseline and after 10-second intervals during the period from 30-180 seconds. The value of flow-mediated dilatation (FMD) in (%) indicates the blood flow increased after release of the sphygmomanometer cuff(28).

The six-minute walking test

The 6MWT was conducted for all patients according ATS guidelines (29). The subjects were instructed to walk at their own pace to cover as much distance as possible for 6 minutes along an enclosed 30-m long corridor in the hospital. Dyspnea and fatigue perception were determined using the modified CR10 Borg Scale (0-10) before and after the 6MWT (30). Heart rate (Polar heart rate monitor, PolarS810i; Polar Electro, Finland), was monitored and oxygen saturation (Risingmed,RMS-50D,Beijing, China) was measured during the test. All patients had previously performed the 6MWT several times so the test was performed only once (31). A Brazilian study was used to calculate the

predicted values of normality (32). Measurements were made with a calibrated Filizola scale (0.1 kg of precision) and with a stadiometer (0.5 cm of precision) and the BMI (body mass kg body height m2) was calculated.

Statistical analyses

Data are expressed as absolute and relative frequency (percentage) for categorical variables or mean and standard deviation for numerical variables. Shapiro-Wilk test was used to assess normality of the data distribution. For comparing continuous variables, a Wilcoxon signed rank sum test was used. We performed a subgroup analysis between patients that received IMT at 50% of MIP and less than 50% of MIP using a Wilcoxon signed rank sum test. Statistical significance was accepted at $P \leq 0.05$. All results were analyzed using commercial software (SPSS ver. 22, SPSS Inc., Chicago, IL, USA; Excel 2010, Microsoft Corporation, Redmond, WA, USA).

Results

The patient's baseline demographic and clinical characteristics and RHC results are summarized on Table 1. All subjects were female and belonged to functional class II of World Health Organization. The hemodynamics parameters showed high mean pulmonary arterial pressure (mPAP) and PVR, but normal cardiac output (CO).

After IMT, the spectral analysis showed significant decrease in the sympathetic modulation and increase in the parasympathetic modulation, seen by LFnu band and HFnu band, respectively. In according to these data, the sympathovagal balance, seen by LF/HF ratio, decreased significantly after exercise. We performed a subgroup analysis between patients that received IMT at 50% of MIP and less than 50% of MIP, and there was no statistical difference with the groups.

Regarding to respiratory muscle strength, the patients had inspiratory muscle weakness in the baseline evaluation, but they reached better values of MIP after the IMT. Moreover, the distance walked in the 6MWT (D6MWT) increased approximately 19 meters (4%).

There was no significant difference in the parameters of endothelial function evaluation after IMT.

Discussion

To our knowledge, this is the first study that showed a significant improvement in the sympathovagal modulation in PH patients after 8 weeks of IMT. This improvement was demonstrated by decrease in sympathetic modulation and increase in parasympathetic modulation, which leaded to a sympathovagal balance reduction.

Data not yet published from our laboratory showed sympathetic modulation predominance in PAH patients, probably due to parasympathetic modulation withdrawal. This sympathetic dominance is important to maintain a compensatory response of cardiovascular system face vascular and respiratory impairment caused by the disease. However, it is clear in the literature that chronic sympathetic overactivation increase the

cardiovascular risks (13-15), thus, this compensatory mechanism would be deleterious for those patients. In the present study, eight weeks of IMT improved the sympathovagal balance by an increase in the parasympathetic and reduction in the sympathetic modulation. This finding is important because reduce the patients cardiovascular risks. Moreover, it is known that the IMT, through of ventilatory muscle strength and endurance improvement, can delay the development of diaphragm fatigue, reduce the sympathetic activation, increase the perfusion of the peripheral muscles, attenuate the exaggerated chemoreflex and improve the ventilatory control and functional capacity (33). Collectively, these findings demonstrate that the respiratory muscle strength improvement facilitates breathing in a general context. We believe that the increase in the respiratory muscle strength probably provides better blood oxygenation, which ultimately, allows a decrease in the compensatory sympathetic response to the cardiopulmonary system.

As reported by Meyer et al, there is reduction in inspiratory and expiratory muscle strength in PAH patients and this weakness occurs independently from ventilatory inefficiency and exercise capacity (11). These findings are in accordance with ours, which showed inspiratory muscle weakness in our patients and also with Saglam et al., who evaluated the IMT in PAH and concluded that this exercise significantly improves inspiratory muscle strength, functional capacity and perception of dyspnea and fatigue (34) . The present study also showed significant improvement in both inspiratory and expiratory muscle strength.

Regarding functional capacity, the 6MWT still is the most used exercise test in PH centers (1). In this study, there was a significant increase in D6MWT after IMT in our subjects. Again, our data is in accordance with Saglan et al, who demonstrated improvement in D6MWT in PAH patients after IMT (an average of 50m in the

intervention group) (34). However, it is important emphasize that the distance of the 6MWT, in absolute values, and not the change, per se, provide prognostic information. There is no single threshold that is applicable for all patients, but it is considered low risk when PAH patient walk distances above 440m (1). However, the increase of D6MWT in the present study could represent an improvement of exercise tolerance caused by respiratory muscle strength increase, as also seen in patients with congestive heart failure (33). In fact, Dall'Ago et al, 2006, found that IMT improved the inspiratory muscle strength and the endurance. According to these authors, this improvement induced also an improvement in the submaximal and maximal functional capacity and quality of life (33).

Regarding to the endothelial dysfunction, our patients have shown similar baseline results to those presented in the literature (6, 7). On the other hand, the IMT did not induce significant improvement in this parameter. We do believe that this absence of difference is probably due to the endothelial response saturation caused by the association with the disease pathophysiology, severity of this disease and the chronic treatment with vasodilators.

There are some limitations to this study: 1-The sample is relatively small, because it is difficult recruit subjects able to perform the training and who met the inclusion criteria. 2-Our patients were treated with different medications that could interfere in their pathophysiology responses; and 3-although all patients belong to groups with similar pathophysiology, the etiology of the PH was not homogeneous.

In conclusion, although the peripheral endothelial function did not show significant difference, the eight weeks of IMT improved sympathetic and parasympathetic balance, the respiratory muscle strength and functional capacity in PH patients, groups I and IV, probably reducing the cardiopulmonary risk.

Disclosures

All authors have read and approved the manuscript and they report no conflicts of interest.

Thisstudy was suported by Santa Casa de Porto Alegre - Pavilhão Pereira Filho, by Universidade Federal de Ciências da Saúde de Porto Alegre, RS, Brazil and by Universidade Federal do Rio Grande do Sul and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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Figure 1

Table 1. Demographic and clinical characteristics of the patients.

Characteristic	N=12
Female	12 (100)
Age (years)	41.2±9.5
BMI (kg/m^2)	26.7±5.3
SBP (mmHg)	115.4±13.4
DBP (mmHg)	75.2±8.8
WHO/NYHA	
Class II	12 (100)
PH etiology	
Congenital heart diseases (corrected)	2 (16.7)
Congenital heart diseases (uncorrected)	2 (16.7)
Connective tissue disease	2 (16.7)
Idiopathic	3 (25.0)
HIV	1 (08.3)
Chronic thromboembolic pulmonary hypertension	2 (16.7)
Drug therapy	
ERA	3 (25.0)
ERA + PD5i	7 (58.3)
sGC	2 (16.7)
Pulmonary function test	
FEV_1(%)	75.7±15.0
FVC (%)	81.8±12.2
FEV_1/FVC (%)	0.76±0.1
Right heart catheterization	
PAPm (mmHg)	47.1±13.6
Cardiac output (L/min)	5.1±1.3
PVR (Wood)	7.8±4.3

Values are n (%) or mean± standard deviation. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WHO, World Health Organization; NYHA, New York Heart Association; PH, pulmonary hypertension; HIV, Human Immunodeficiency Virus; ERA, endothelin receptor antagonist; PD5i, phosphodiesterase type 5 inhibitor; sCG, soluble guanylyl cyclase; FEV_1, forced expiratory volume of 1 second; FVC, forced volume vital capacity; FEV_1/FVC ratio, Tiffeneau-Pinelli index; PAPm, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance.

Figure 2

Table 2. Effects of inspiratory muscle training (IMT)

Variables	Baseline	After IMT	P value
Respiratory muscle strength			
PIP, cmH_2O	66.5±6	111.7±21	0.002
PEP, cmH_2O	79.4±20	103.7±19	0.002
Six-minute walking test			
Distance, m	500±65	519±56	0.030
Distance, % predicted	87±11	89±10	0.200
Spectral analysis			
HR, bpm	71±10	68±7	0.200
HRV, ms^2	923±546	885±417	0.760
LFpeak, Hz	0.05±0.01	0.04±0.004	0.620
HFpeak, Hz	0.25±0.1	0.3±0.091	0.280
LF band, ms^2	238±190	214±162	0.760
HF band, ms^2	217±189	242±189	0.500
LF band, nu	59±21	49±17	0.048
HF band, nu	41±21	51±17	0.048
LF/HF ratio	2.0±1.4	1.2±0.8	0.030
Endothelial function			
B-DIA, mm	317±40	314±52	0.460
RH-DIA, mm	327±33	324±43	0.900
FMD, %	3.2±4	4.1±5	0.700

Note: PIP, peak inspiratory pressure; PEP, peak expiratory pressure; HR, heart rate; HRV, heart rate variability; LF, low-frequency component; HF, high-frequency component; LF/HF, ratio between low- and high-frequency power components; bpm, beats per minute; nu, normalized units; B-DIA, basal brachial artery diameter; RH-DIA, brachial artery diameter with reactive hyperemia; FMD, flow-mediated dilation; $P < 0.05$ was considered statistically significant.

5 CONCLUSÃO

A avaliação basal dos sujeitos do estudo mostrou modulação parassimpática reduzida, predomínio da modulação simpática em detrimento da parassimpática e consequente desbalanço simpatovagal. Além disso, os pacientes apresentaram disfunção endotelial periférica que não se correlacionou com a capacidade funcional ou modulação simpatovagal. Já a modulação parassimpática reduzida e o consequente desbalanço simpatovagal correlacionaram-se, de maneira inversa, com a capacidade funcional dos pacientes.

No entanto, após 8 semanas de IMT, apesar de não haver diferença na função endotelial, houve melhora significativa no balanço simpatovagal, provavelmente reduzindo o risco cardiovascular nestes pacientes com hipertensão pulmonar dos grupos I e IV.

6 ANEXOS

ANEXO A - Termo De Consentimento Livre E Esclarecido

TERMO DE CONSENTIMENTO LIVRE ESCLARECIDO

TÍTULO DO ESTUDO: "**Estudo do efeito do treinamento muscular inspiratório sobre a função endotelial, controle autonômico, capacidade de exercício e qualidade de vida em pacientes com hipertensão arterial pulmonar**".

INVESTIGADOR PRINCIPAL: Fabrício Farias da Fontoura

INSTITUIÇÃO: Irmandade Santa Casa de Misericórdia de Porto Alegre

TELEFONE: (51) 3213 7068

INFORMAÇÕES GERAIS

Você está sendo convidado a participar de um estudo para pacientes com Hipertensão Arterial Pulmonar (HAP). A participação neste estudo é totalmente voluntária. Antes de decidir a respeito de sua participação, você receberá algumas informações para compreender o estudo e poder tomar sua decisão. Este documento é denominado Termo de Consentimentos Livre e Esclarecido (TCLE), contém todas as informações sobre o estudo, seus objetivos, benefícios, desconfortos e precauções. Você poderá sair do estudo a qualquer momento sem haver qualquer prejuízo em relação a seu médico e equipe de saúde responsável pelo seu cuidado.

As informações abaixo deverão ser lidas e apenas quando você entender e decidir sobre sua participação deverá assinar duas vias deste documento. Uma cópia ficará com o médico responsável pelo estudo e outra ficará com você.

DESCRIÇÃO DO ESTUDO

Este estudo tem como objetivo investigar os efeitos treinamento de força muscular ventilatória obre a função endotelial, o balanço autonômico, a capacidade de exercício e qualidade de vida em pacientes com HAP.

Concordando em participar deste estudo, você inicialmente realizará as seguintes avaliações (exames): Eletrocardiograma, ultrassonografia, teste de caminhada de seis minutos, avaliação de força muscular diafragmática, teste em bicicleta ergométrica com carga constante, teste cardiopulmonar incremental em bicicleta ergométrica e questionário de qualidade de vida.

Após estas avaliações, você será sorteado para participar de um dos dois grupos que são: Grupo I, onde você realizará treinamento muscular inspiratório domiciliar diário e uma vez por semana ambulatorial com uma carga de 50% da força máxima ou Grupo II, onde você irá realizar o mesmo treinamento exceto a carga, que será reduzida para 02% da força máxima.

Durante os exercícios ambulatoriais você será constantemente supervisionado e monitorado e questionado quanto ao seu cansaço, desconforto ou falta de ar, e poderá interromper os exercícios sempre que achar necessário. Você receberá da equipe um treinamento para usar um dispositivo chamado *power breathe* (válvula para puxar o ar) a fim de realizar o treinamento muscular ventilatório domiciliar, o mesmo deverá ser devolvido a equipe ao final do estudo.

Após as 08 e 16 semanas do início do estudo, você realizará novamente todas as avaliações realizadas anteriormente, tais como: Eletrocardiograma, ultrassonografia, teste de caminhada de seis minutos, avaliação de força muscular diafragmática, teste em bicicleta ergométrica com carga constante e questionário de qualidade de vida.

A medicação prescrita por seu médico para o tratamento da HAP deverá ser mantida, exceto se o médico orientar alguma modificação, quando realizada deve ser comunicado a equipe de pesquisa.

Você poderá mudar de idéia em relação à sua participação no estudo a qualquer momento e por qualquer razão que julgar correta. Basta comunicar sobre sua desistência, sem a necessidade de se justificar. Da mesma forma, se for da sua vontade, você poderá solicitar o resultado dos exames realizados nesta pesquisa.

RISCOS

Embora a literatura descreva que não ocorreram eventos adversos nos estudos que submeteram pacientes com hipertensão pulmonar arterial em programas de exercício físico supervisionado ou não, o risco sempre é existente. Poderão acorrer desmaios, aumento da falta de ar, cansaço muscular e tonturas. Para isso você será constantemente monitorado e indagado sobre percepção de esforço a fim de evitar possíveis eventos adversos.

BENEFÍCIOS

Os pacientes de ambos os grupos serão beneficiados com melhora na capacidade funcional e melhora na qualidade de vida. Caso exista uma diferença clinicamente significativa entre o grupo I e II, será oferecida a intervenção com melhor desfecho aos pacientes que queiram participar após o termino do estudo.

DESPESAS E PAGAMENTOS

Você não receberá nenhum pagamento e nem terá nenhuma despesa por participar deste estudo.

CONFIDENCIALIDADE DOS DADOS

A privacidade de quem participa de pesquisas é muito importante. Todas as informações coletadas neste estudo serão mantidas confidenciais. Caso os resultados venham a ser publicados, sua identidade será preservada. Os registros médicos que possam identificar você serão mantidos em sigilo, conforme exigido por lei. Exceto pelo nome neste documento, que também é confidencial, você não será identificado pelo número de RG ou CPF, endereço, número de telefone ou qualquer outro dado que o identifique diretamente nos registros do estudo que forem revelados para fora da Santa Casa de Misericórdia de Porto Alegre.

TÉRMINO DO ESTUDO

Você poderá ser afastado da pesquisa pelas seguintes razões: se o médico julgar que os efeitos são perigosos para sua saúde, e trazem mais riscos do que os benefícios previstos; se você faltar muitas vezes às sessões; se interromper seu tratamento medicamentoso para HAP sem consentimento do seu médico; se for do sexo feminino e ficar grávida durante o estudo.

INFORMAÇÕES DE CONTATO

Se houver qualquer problema de saúde referente ao estudo durante sua participação entre em contato com o pesquisador responsável Fabrício Farias da Fontoura, no telefone: (51) 3213-7068. Ele deverá tomar as devidas providências.

Se você tiver alguma dúvida ou consideração sobre as questões éticas, entre em contato com o Comitê de Ética em Pesquisa (CEP) da Santa Casa de Misericórdia de Porto Alegre, situado na Rua Professor Annes Dias, 295 – HHospital Dom Vicente – 6º andar – Porto Alegre/RS, fone: (51) 3214-8571.

CONSENTIMENTO DO PACIENTE

Eu confirmo que li e compreendi a descrição do "Estudo do efeito do treinamento muscular inspiratório sobre a função endotelial, controle autonômico, capacidade de exercício e qualidade de vida em pacientes com hipertensão arterial pulmonar", e discuti com o pesquisador responsável até esgotar as minhas dúvidas no momento. Compreendo que a minha participação no estudo é totalmente voluntária. Estou ciente do propósito, dos procedimentos, dos riscos e dos benefícios do estudo, desta forma autorizo que o(s) procedimento(s) seja (m) realizado (s) da forma como foi (foram) exposto(s) no presente estudo. Esta autorização é dada ao(à) pesquisador responsável(a) bem como ao(s) membro(s) de sua equipe e/ou outro(s) profissional(ais) por ele (ela) selecionado(s) a intervir no(s) procedimento(s) de acordo com seu julgamento profissional quanto à necessidade de participação do(s) mesmo(s) no estudo. Estou também ciente que posso desistir do estudo a qualquer momento, sem que meu tratamento seja afetado. Declaro que recebi uma cópia deste Termo de Consentimento Livre Esclarecido.

_____ - _____

Nome do Paciente Data

_____

Assinatura do Paciente

_____ - _____

Nome do pesquisador que explicou o TCLE Data

_____

Assinatura do pesquisador que explicou o TCLE

_____ - _____

Testemunha (se aplicável) Data

_____

Assinatura da testemunha

ANEXO B - Parecer de Aprovação do Comitê de Ética da Santa Casa De Misericórdia de Porto Alegre

IRMANDADE DA SANTA CASA DE MISERICORDIA DE PORTO ALEGRE - ISCMPA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Estudo do efeito do treinamento muscular inspiratório sobre a função endotelial, controle autonômico, capacidade de exercício e qualidade de vida em pacientes com hipertensão arterial pulmonar

Pesquisador: FABRICIO FARIAS DA FONTOURA

Área Temática:

Versão: 1

CAAE: 30199714.6.0000.5335

Instituição Proponente: ISCMPA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 648.580

Data da Relatoria: 14/05/2014

Apresentação do Projeto:

Descrito no Parecer Consubstanciado do CEP 646.570.

Objetivo da Pesquisa:

Descrito no Parecer Consubstanciado do CEP 646.570.

Avaliação dos Riscos e Benefícios:

Adequados e já descritos no Parecer Consubstanciado do CEP 646.570.

Comentários e Considerações sobre a Pesquisa:

Adequados.

Considerações sobre os Termos de apresentação obrigatória:

Conforme solicitado no Parecer Consubstanciado do CEP 646.570, as pendências foram todas atendidas e anexadas.

Recomendações:

Não se aplica.

Conclusões ou Pendências e Lista de Inadequações:

Não se aplica.

Endereço: R. Profº Annes Dias,285 Hosp.Dom Vicente Scherer
Bairro: 6º andar - Centro **CEP:** 90.020-090
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3214-8571 **Fax:** (51)3214-8571 **E-mail:** cep@santacasa.tche.br

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Considerações Finais a critério do CEP:
Após reavaliação do protocolo acima descrito, o presente comitê não encontrou óbices quanto ao desenvolvimento do estudo em nossa Instituição e poderá ser iniciado a partir da data deste parecer.

Obs.: 1 - O pesquisador responsável deve encaminhar à este CEP, Relatórios de Andamento dos Projetos desenvolvidos na ISCMPA. Relatórios Parciais (pesquisas com duração superior à 6 meses), Relatórios Finais (ao término da pesquisa) e os Resultados Obtidos (cópia da publicação).

2 – Para o início do projeto de pesquisa, o investigador deverá apresentar a chefia do serviço (onde será realizada a pesquisa), o Parecer Consubstanciado de aprovação do protocolo pelo Comitê de Ética.

PORTO ALEGRE, 15 de Maio de 2014

Assinado por:
Claudio Teloken
(Coordenador)

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ANEXO C - Parecer de Aprovação do Comitê de Ética da Universidade Federal de Ciências da Saúde de Porto Alegre

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Estudo do efeito do treinamento muscular inspiratório sobre a função endotelial, controle autonômico, capacidade de exercício e qualidade de vida em pacientes com hipertensão arterial pulmonar

Pesquisador: FABRICIO FARIAS DA FONTOURA

Área Temática:

Versão: 1

CAAE: 30199714.6.3001.5345

Instituição Proponente: ISCMPA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 730.142

Data da Relatoria: 17/07/2014

Apresentação do Projeto:

Ensaio clínico randomizado controlado por placebo com objetivo de investigar os efeitos do treinamento da musculatura inspiratória (TMI) sobre afunção endotelial, função autonômica, capacidade de exercício e qualidade de vida em pacientes com hipertensão arterial pulmonar. Para realizaçãodo estudo serão recrutados 32 pacientes provenientes do serviço de Hipertensão Pulmonar do Hospital Dom Vicente Scherer, divididos em 2 grupos

(n=16 em cada) de treinamento muscular inspiratório, Grupo I - 30% da carga máxima e Grupo II - 02% da carga máxima. Os sujeitos do estudoserão avaliados com testes padronizados validados antes de iniciar a intervenção, 8 semanas após a intervenção e 8 semanas após o final daintervenção. Os procedimentos serão realizados no Serviço de Reabilitação Pulmonar do Pavilhão Pereira Filho e no laboratório de reabilitação

pulmonar da Universidade Federal de Ciências da Saúde de Porto Alegre. O período do estudo será de Abril de 2014 a Junho de 2015, envolvendoos participantes na primeira fase de 2 meses (1 visita semanal) e na segunda fase após 16 semanas do início do estudo (três dias de reavaliações

Objetivo da Pesquisa:

Objetivo Primário:

Investigar os efeitos do treinamento da musculatura inspiratória (TMI) sobre a função endotelial,

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função autonômica, capacidade de exercício e
qualidade de vida em pacientes com hipertensão arterial pulmonar.
Objetivo Secundário:
Avaliar as seguintes variáveis antes e após a intervenção: força muscular inspiratória; capacidade submáxima de exercício; capacidade máxima deexercício; tolerância ao exercício; qualidade de vida; nível de atividade física; função endotelial; função do sistema nervoso autonômico.

Avaliação dos Riscos e Benefícios:
Riscos:
Poderão acorrer síncopes, aumento da dispneia, fadiga muscular e tonturas. Para isso os pacientes serão constantemente monitorados e indagados
sobre percepção de esforço afim de evitar possíveis eventos adversos.
Benefícios:
Os pacientes dos dois grupo serão beneficiados com melhora na capacidade funcional e melhora na qualidade de vida segundo alguns estudosprévios. Caso exista uma diferença clinicamente significativa entre o grupo I e II será oferecido a mesma intervenção aos pacientes que queiramparticipar após o termino do estudo.

Comentários e Considerações sobre a Pesquisa:
Projeto já aprovado no CEP da Santa Casa - instituição proponente

Considerações sobre os Termos de apresentação obrigatória:
adequados

Recomendações:
aprovar

Conclusões ou Pendências e Lista de Inadequações:
não há inadequações ou pend^rncias

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Considerações Finais a critério do CEP:
De acordo com o parecer do relator.

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PORTO ALEGRE, 28 de Julho de 2014

Assinado por:
José Geraldo Vernet Taborda
(Coordenador)

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ANEXO D – Normas das Revistas Científicas

Nome da revista: ***Respiratory Physiology & Neurobiology***

Author information

Respiratory Physiology & Neurobiology publishes original articles and invited reviews concerning the field of respiration in its broadest sense. Although a special focus is on topics in neurobiology, high quality papers in respiratory molecular and cellular biology are also welcome, as are high quality papers in traditional areas, such as mechanics of breathing; gas exchange in lungs, gills, skin, and tissues; acid-base balance; respiration at rest and exercise; respiration in normal and unusual conditions, like high or low pressure or changes of temperature, low ambient oxygen; embryonic and adult respiration; comparative respiratory physiology. Papers on clinical aspects, articles on original methods, as well as theoretical papers are also considered as long as they foster the understanding of respiratory physiology.

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Abstract Main text References Figure legends (if any)

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2) Opie LH. Mechanism of cardiac contraction and relaxation. In: Braunwald E, editor. Heart disease, 5th edn. Philadelphia: Saunders, 1997; 360–393.

3) Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, et al. 2009 Focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults A Report of the ACC/AHA Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. J Am CollCardiol 2009; 53: e1 - e90, doi:10.1016/j.jacc.2008.11.013.

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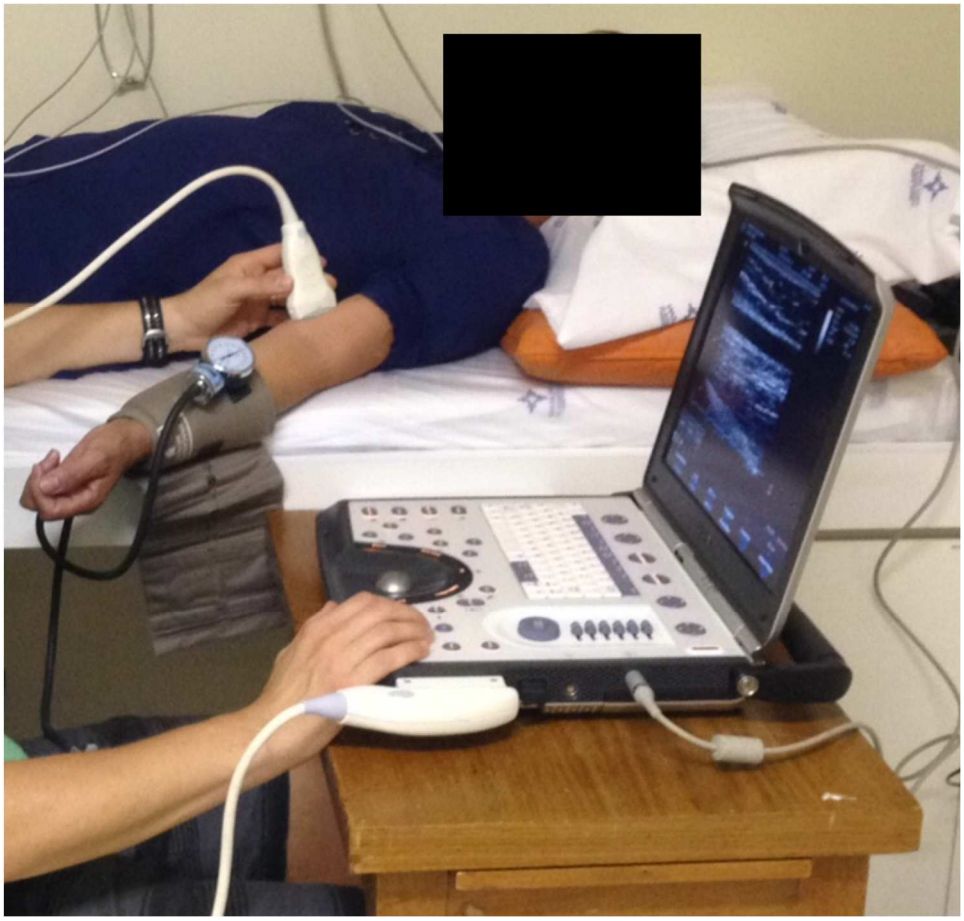
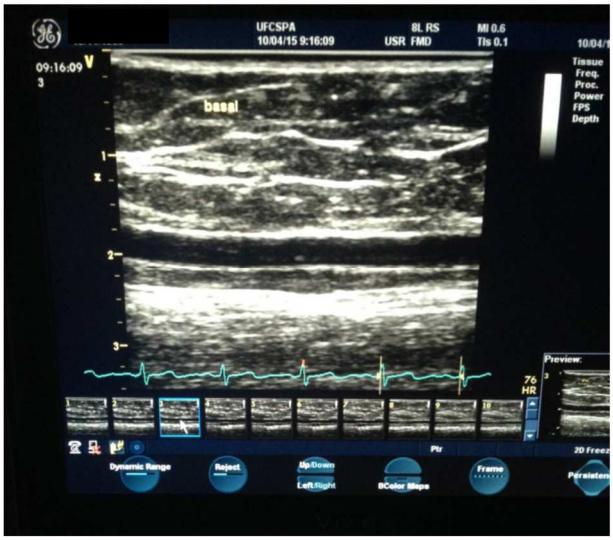
A) Exame de ultrassonografia da artéria braquialpara avaliação da função endotelial sendo realizado.

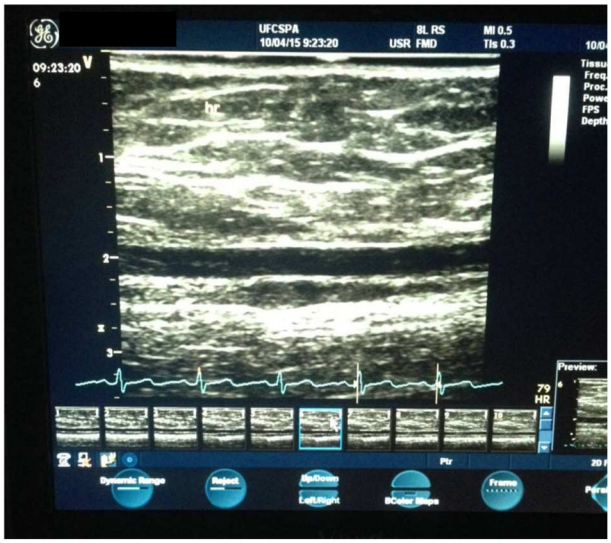


ANEXO F – Imagensde ultrassonografia da artéria braquial

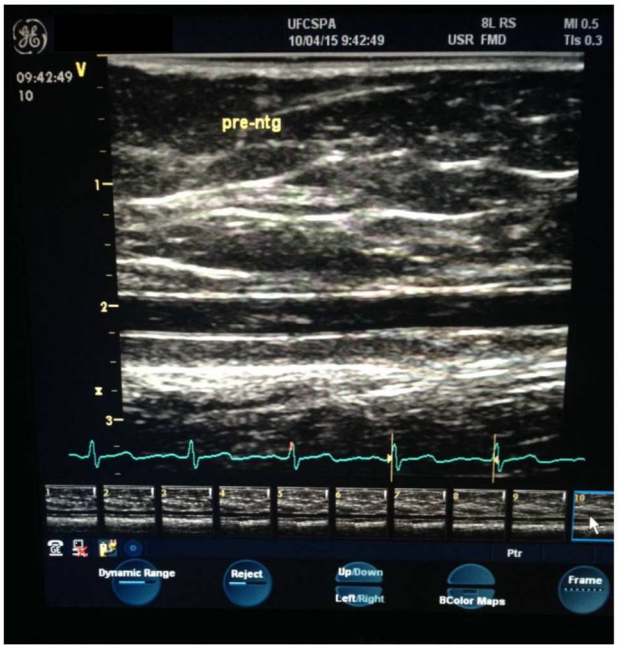
A) Imagem da ultrassonografia da avaliação basal da artéria braquial.



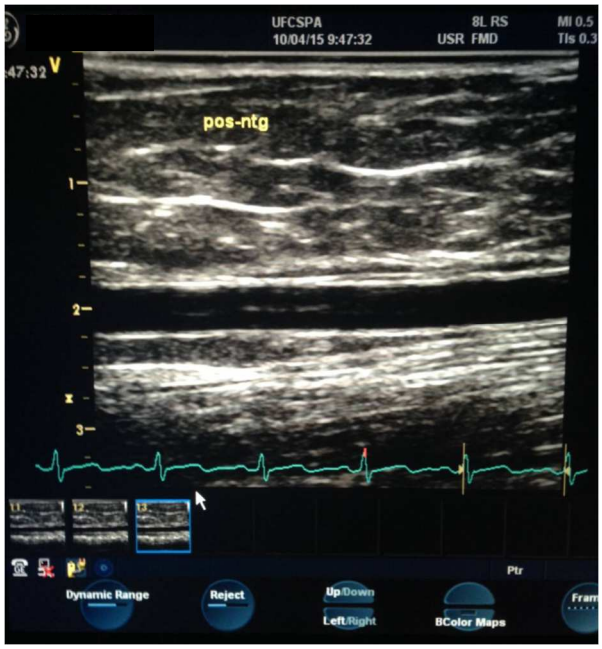
B) Imagem da ultrassonografia da avaliação da artéria braquial pós-hiperemia reativa.



C) Imagem da ultrassonografia da avaliação da artéria braquial previa à administração da nitroglicerina.



D) Imagem da ultrassonografia da avaliação da artéria braquial após a administração de nitroglicerina



ANEXO G –Teste de esforço cardiopulmonar

A) Paciente realizando exame de CPET com supervisão.

B) Equipe clínica realizando monitoramento e avaliação do CPET.