UNIVERSIDADE ESTADUAL DE CAMPINAS
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"EFEITOS DO TREINAMENTO FÍSICO NA REMODELAÇÃO CARDÍACA E NO MÚSCULO ESQUELÉTICO DURANTE A TRANSIÇÃO ENTRE DISFUNÇÃO VENTRICULAR E INSUFICIÊNCIA CARDÍACA"

Tese apresentada ao Instituto de Biologia para obtenção do Título de Doutor em Biologia Celular e Estrutural, na área de Biologia Celular.

Orientadora: Profa. Dra. Maeli Dal Pai Silva

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Dedico este trabalho....

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Sumário

Lista de Abreviaturas

AS: aortic stenosis
AS18: aortic stenosis 18 weeks
AS28: aortic stenosis 28 weeks control
ASTR aortic stenosis 28 weeks training
ATs: atrium
C18: control 18 weeks/ controle 18 semanas
C28: control 28 weeks/ controle 28 semanas
CLFS- chronic low-frequency stimulation
CR: cardiac remodeling
CS: citrate synthase/ citrato sintase
CTR: controle 28 semanas treinado
E/A: E wave mitral flow; A wave mitral flow
EAo: estenose aórtica
EAo18: estenose aórtica 18 semanas
EAo28: estenose aórtica 28 semanas
EAoTR: estenose aórtica 28 semanas treinado
EDL: extensor longo dos dedos
EFS: endocardial fractional shortening
FBW: final body weight
HF: heart failure
IC: insuficiência cardíaca
IGF-I: insulin-like growth factor
ISV: interventricular septum thickness;
LA/Ao: left atrium/aorta.
LV: left ventricular
LVDD: left ventricular diastolic dimension;
LVSD: ventricular systolic dimension;
LVWT: left ventricular posterior wall thickness;
MHC: myosin heavy chain/ miosinas de cadeia pesada

MRFs: myogenic regulatory factors/fatores de regulação miogênica

PT: physical training

PWSV: posterior wall shortening velocity;

RC: remodelação cardíaca

RV: right ventricular

Sol: soleus

TLV: total left ventricular

TR: control 28 weeks training

TR: treinamento físico

1. RESUMO

Introdução: A sobrecarga pressórica imposta pela estenose da valva aórtica (EAo) progride para disfunção ventricular e Insuficiência Cardíaca (IC). Na IC ocorre Remodelação Cardíaca (RC) e mudanças dos tipos de fibras do músculo esquelético. Os mecanismos moleculares que são responsáveis pelas alterações das fibras musculares na IC ainda não foram descritos. Os fatores de regulação miogênica (MRF), uma família de fatores transcricionais que controlam vários genes músculo-específicos, podem estar relacionados com essa miopatia. Entre os MRFs, a MyoD está relacionada com aumento do TNF-α e diminuição do IGF-I e a miogenina com o metabolismo oxidativo. Estudos têm enfatizado os efeitos do Treinamento Físico (TF) sobre a RC e músculo esquelético na IC. A hipótese deste estudo é que a IC pode alterar os MRFs e que a aplicação do TF antes de se instalar a IC promoverá melhora na RC e reverterão às alterações fenotípicas do músculo esquelético, os MRFs e seus possíveis mecanismos de controle.

Objetivos: Avaliar os efeitos do TF durante a transição entre disfunção ventricular e IC induzida por EAo pela avaliação da RC e do músculo estriado esquelético.

Métodos: Após 18 semanas de EAo, quando os animais apresentaram disfunção ventricular estes foram submetidos a um TF durante 10 semanas em uma esteira. A IC foi avaliada por parâmetros clínicos e a RC por dados morfológicos e ecocardiograma. Foram avaliados no músculo soleus os tipos de fibras, as miosinas de cadeia pesada (MHC), a atividade da Citrato Sintase (CS), a expressão gênica e protéica do IGF-I, MyoD e miogenina e os níveis séricos de TNF- α.

Resultados: O grupo EAo28 apresentou sinais de IC (taquipnéia, ascite, trombo em átrio esquerdo, derrame pleural) e o grupo EAoTF apresentou diminuição da intensidade destes. A RC foi amenizada com o TF e ocorreu aumento da porcentagem de encurtamento do endocárdio (EAo28=40,90 ± 16,18% vs. EAoTF=52,27 ± 11,60%) e velocidade de encurtamento da parede posterior (EAo28=24,31 ± 6,05mm/s vs. EAoTF=30,96 ± 3,94mm/s). Os diâmetros sistólicos e diastólicos (EAo28=5,70 ± 1,95mm vs. EAoTF=4,03 ± 1,37mm; EAo28=9,33 ± 1,30mm vs. EAoTF=8,27 ± 1,03mm, respectivamente), as relações diâmetro do átrio esquerdo/diâmetro da aorta (EAo28=2,18±0,28mm vs. EAoTF=1,88±0,26mm) e ondas E/A mitral (EAo28=5,20 ± 3,02 vs. EAoTF=2,86 ± 2,84) diminuíram. O TF alterou o fenótipo muscular com aumento das fibras do tipo I (EAo28=8,47 ± 6,02% vs. EAoTF=13,0 ± 6,11%) e diminuição das fibras do tipo IIa no EAoTR em relação ao EAo28 (EAo28=22,08 ± 10,28% vs. EAoTF=13,95 ± 2,94%). Não houve alterações nos níveis de TNF- α, na atividade da CS, na expressão gênica e protéica do IGF-I, MyoD e miogenina na IC e após o TF.

Conclusão: O TF melhorou a RC, diminuiu os sinais clínicos da IC e reverteu às alterações fenotípicas do músculo soleus, sem alterar os MRFs, TNF-α e metabolismo oxidativo durante a transição entre disfunção ventricular e IC em ratos com estenose aórtica. Os MRFs parecem não estar relacionados à modulação do fenótipo muscular e a sua reversão pelo TF na EAo.

2. ABSTRACT

Background: Pressure overload imposed by aortic valve stenosis (AS) progresses to ventricular dysfunction and heart failure (HF). In HF occurs Cardiac Remodeling (CR) and changes in fiber types of skeletal muscle. The molecular mechanisms that are responsible for changes in muscle fibers in HF has not been described. The myogenic regulatory factors (MRF), a family of transcriptional factors that control several muscle-specific genes, may be associated with this myopathy. Among the MRFs, MyoD is related to increase of TNF-α and decrease of IGF-I and myogenin with oxidative metabolism. Studies have emphasized the effects of physical training (PT) on the CR and skeletal muscle in HF. Our hypothesis is that the HF can change the MRFs and the application of the PT before installing the HF will promote improvement in CR and revert the phenotypic changes of skeletal muscle, the MRFs and their possible control mechanisms.

Objectives: Evaluate the effects of PT during the transition between ventricular dysfunction and HF induced by AS through assessment of CR and skeletal muscle.

Methods: After 18 weeks of AS, when the animals presented ventricular dysfunction, they were submitted to a PT on a treadmill during 10 weeks. HF was evaluated by clinical parameters and, the CR, by morphological data and echocardiogram. We evaluated fiber types in soleus muscle, the myosin heavy chain (MHC), the activity of citrate synthase (CS), the gene and protein expression of IGF-I, MyoD and myogenin and the serum levels of TNF-α.

Results: The AS28 group presented signs of HF (tachypnea, ascites, thrombus in left atrium, pleural effusion) and ASPT group presented reduced intensity of these. CR was

reduced with the PT and it increased the percentage of shortening of the endocardium (AS28=40,90 ± 16,18% vs. ASPT=52,27 ± 11,60%) and shortening velocity of the posterior wall (AS28=24,31 ± 6,05 mm/s vs. ASPT=30,96 ± 3,94 mm/s). The systolic and diastolic diameters (AS28=5,70 ± 1,95 mm vs. ASPT=4,03 ± 1,37 mm; AS28=9,33 ± 1,30 mm vs. ASPT=8,27 ± 1,03 mm, respectively) relations of left atrium diameter/aortic diameter (AS28=2,18 ± 0,28 mm vs. ASPT= 1,88 ± 0,26 mm) and E/A wave mitral (AS28=5,20 ± 3,02 vs. ASPT=2,86 ± 2,84) decreased. The PT changed the muscle phenotype increasing fiber type I (AS28= 8,47 ± 6,02% vs. ASPT=13,0 ± 6,11%) and decreasing fibers type IIa in ASPT in relation to AS28 (AS28=22,08 ± 10,28% vs. ASPT=13,95 ± 2,94%). There were no changes in levels of TNF-α in CS activity, gene expression and protein of IGF-I, MyoD and myogenin in HF and after PT.

Conclusion: PT improved CR, decreased clinical signs of HF and reversed the phenotypic changes of the soleus muscle without altering the MRFs, TNF-α and oxidative metabolism during the transition between ventricular dysfunction and HF in rats with aortic stenosis. The MRFs seems not to be related to the modulation of muscle phenotype and its reversal by PT in the AS.

3. INTRODUÇÃO

A Insuficiência Cardíaca (IC) constitui um importante problema clínico devido à gravidade de suas manifestações e à sua grande prevalência. No Brasil não existem estudos epidemiológicos envolvendo a incidência de insuficiência cardíaca. Porém, de acordo com outros países pode-se estimar que até 6,4 milhões de brasileiros sofram de insuficiência cardíaca (Guimarães et al., 2002). A IC encontra-se entre as principais causas de internação do Sistema Único de Saúde, a partir dos 65 anos (Albanesi Filho, 1998; Rossi Neto, 2004). A prevalência da insuficiência cardíaca está em ascenção, em decorrência do incremento na expectativa de vida de nossa população e maior efetividade dos novos medicamentos para o tratamento, prolongando a vida.

Entre seus sintomas da IC encontram-se a dispnéia e o cansaço associados à diminuição da tolerância aos esforços e piora da qualidade de vida. Medidas não farmacológicas como o treinamento físico têm sido propostas para minimizar as conseqüências desta patologia.

Em pacientes com insuficiência cardíaca os estudos sobre o custo-efetividade do tratamento por meio da Reabilitação Cardiopulmonar e Metabólica (treinamento físico) têm mostrado resultados expressivos com recomendação grau A (baseado em muitos estudos randomizados, controlados) e nível 1 de evidência (recomendação conclusiva, sempre devem ser indicados) (Guimarães, 2006).

Porém, são necessários mais estudos que avaliem e fundamentem na insuficiência cardíaca induzida por estenose da valva aórtica, os benefícios do treinamento físico na remodelação cardíaca e nos mecanismos moleculares envolvidos no músculo esquelético (fatores de regulação miogênicos, tipos de fibras musculares, metabolismo e mediadores inflamatórios).

Estes estudos possibilitarão uma maior compreensão deste tipo de intervenção (treinamento físico) na IC, o que contribuirá diretamente para uma melhor qualidade de vida destes pacientes.

3.1. Músculo Estriado Esquelético e Fatores de Regulação Miogênica (MRFs)

A regulação do processo de formação dos músculos esqueléticos envolve a apropriada ativação, proliferação e diferenciação de linhagens de células miogênicas e depende da expressão e atividade de fatores transcricionais, conhecidos como fatores de regulação miogênica (MRFs).

Durante o desenvolvimento embrionário, o comprometimento das células somíticas do mesoderma com a linhagem miogênica depende inicialmente de sinais positivos [Wnts, Sonic hedgehog, Noggin] ou negativos (BMP4) oriundos de tecidos circundantes, tais como a notocorda e o tubo neural (revisado em Chargé & Rudnicki, 2004). Esses sinais irão ativar os genes capazes de transformar células não musculares em células com um fenótipo muscular.

Os genes responsáveis por essa transformação são membros da família dos fatores transcricionais “basic helix-loop-helix” (bHLH), da qual fazem parte a MyoD, Miogenina, Myf5 e o MRF4, coletivamente chamados de fatores de regulação miogênica (do inglês, myogenic regulatory factors ou MRFs). Os MRFs compartilham um domínio homólogo bHLH, que é necessário para a ligação com o DNA e para a dimerização com fatores transcricionais da família da proteína E (Patapoutian et al., 1995; Rawls et al., 1995; Zhang et al., 1995, Yoon et al., 1997).

Os heterodímeros MRF-proteína E e os monômeros de MRFs ligam-se a seqüências de DNA (5´-CANNTG-3´) conhecidas como Ebox, presentes na região promotora de vários genes músculo – específicos, levando à expressão dos mesmos (Murre et al., 1989; Lassar et al., 1991) (Figura 1).

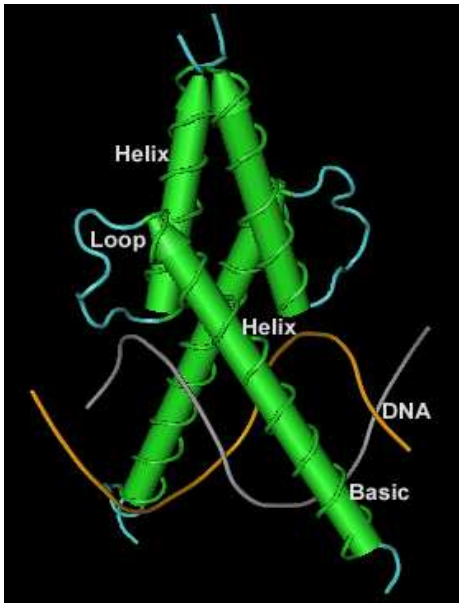

Figura 1. Estrutura cristalográfica do complexo formado pelo dímero do fator transcricional da família "basic Helix-Loop-Helix" (bHLH) MyoD e o DNA (adaptado de Ma et al., 1994).

Assim como os MRFs, a família de fatores transcricionais MEF2 (do inglês, myocyte enhancer factor-2) também está envolvida na ativação de genes músculo - específicos (revisado em Naya & Olson, 1999). Os MEF2 são expressos em muitos tecidos, mas é apenas durante o desenvolvimento dos músculos cardíaco, liso e estriado que esses fatores ativam a transcrição (Naya et al., 1999). Estudos demonstram uma ação interdependente entre a família MEF2 e os MRFs no controle da diferenciação do músculo esquelético (Naidu et al., 1995; Novitch et al., 1996; Novitch et al., 1999; Ridgeway et al., 2000)

Na diferenciação do músculo esquelético, o comprometimento das células somíticas do mesoderma com a linhagem miogênica é marcado pela expressão dos MRFs Myf5 e MyoD (Figura 2). Isso é demonstrado pela total ausência de tecido muscular em camundongos duplo Knockout MyoD:Myf5 e pela observação de que, nesses animais, as supostas células progenitoras musculares permanecem multipotentes e contribuem para tecidos não musculares do tronco e dos membros desses camundongos. Estes animais são imóveis e morrem após o nascimento (Rudnicki et al., 1993; Kablar et al 1998; Palmer & Rudnicki, 2001). As células da

linhagem miogênica em proliferação, positivas para Myf5 e/ou MyoD, são então denominadas de mioblastos (Megeney & Rudnicki 1995).

Embora a MyoD e o Myf5 definam a identidade dos mioblastos, as células precursoras somíticas devem ser “pré-comprometidas” com a linhagem miogênica antes da expressão dos MRFs. No embrião, esse “pré-comprometimento” é realizado pelo fator transcricional Pax3, da família Pax (do inglês, paired-box), o qual é expresso em células do mesoderma pré-somítico e dos primeiros somitos epiteliais (Goulding et al., 1994; Williams & Ordahl, 1994). Já no dermomiótomo, as células precursoras, que apresentam expressão de Pax3 induzida por sinais secretados pelo mesoderma da placa lateral e pelo ectoderma superficial, são mantidas como uma população não diferenciada e em proliferação, contribuindo assim para a expansão das células da linhagem miogênica (Amthor et al., 1999) (Figura 2).

Os mioblastos que saem do ciclo celular, positivos para Myf5 e MyoD, tornam-se miócitos diferenciados e iniciam a expressão dos MRFs miogenina e MRF4, os quais regulam a diferenciação dessas células em fibras musculares (Figura 2) (Megeney & Rudnicki 1995). Embriões deficientes em miogenina morrem no período perinatal devido à deficiência na diferenciação dos miócitos, evidenciada pela quase total ausência de fibras musculares nesses mutantes (Hasty et al., 1993; Nabeshina et al., 1993).

Finalmente, no processo de miogênese, os miócitos mononucleados se fundem para formar os miotubos (Figura 2) e, no animal adulto, o músculo esquelético é caracterizado por fibras musculares multinucleadas (Decary et al., 1997; Schmalbruch & Lewis, 2000).

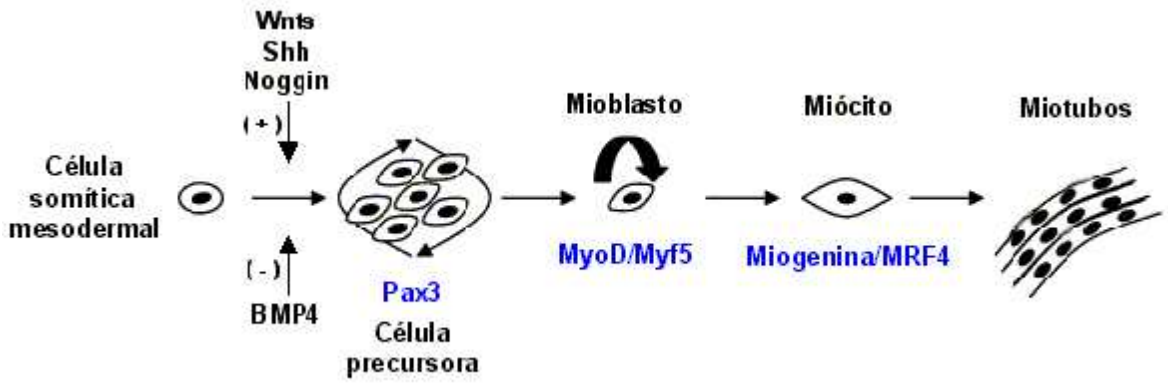

Figura 2. Células somíticas mesodermais recebem sinais de tecidos circundantes os quais podem induzir [Wnts, Sonic hedgehog (Shh), Noggin] ou inibir (BMP4) a expressão de Myf5 e MyoD. A expressão de Pax3 nas células precursoras contribui para a expansão das células miogênicas. Após a indução de Myf5 e/ou MyoD, as células somíticas mesodermais são comprometidas com a linhagem miogênica (mioblastos). A expressão de miogenina e MRF4 induz a diferenciação dos mioblastos em miócitos. Posteriormente, os miócitos se fundem para originar os miotubos.

3.2 Características contráteis das fibras musculares esqueléticas adultas

Os primeiros estudos envolvendo o tecido muscular classificavam os músculos em “vermelhos” ou “brancos” (Ranvier, 1873). A cor vermelha está relacionada com a presença do pigmento mioglobina e com o grau de vascularização do músculo. Com a utilização de técnicas histoquímicas, observou-se que a maioria dos músculos estriados dos mamíferos é constituída por uma população heterogênea de fibras, que apresentam características morfológicas, bioquímicas e fisiológicas distintas (Dubowitz & Pearse, 1960). Inicialmente, as fibras musculares foram classificadas em vermelhas, intermediárias e brancas (Ogata, 1958). Posteriormente, três tipos principais de fibras musculares foram descritas, sendo denominadas de fibras dos tipos I, IIA e IIB, de acordo com o padrão de reação para a atividade da ATPase da porção globular da cadeia pesada da miosina (ATPase miofibrilar ou m-ATPase) (Brooke & Kaiser, 1970).

A molécula de miosina é um hexâmero formado por duas cadeias pesadas de miosina (do inglês, myosin heavy chain ou MHC), enroladas em α-hélice, e quatro cadeias leves de miosina (do inglês, myosin light chain ou MLC) (Lowey et al. 1969; Weeds & Lowey, 1971; Elliot & Offer, 1978; Warrick & Spudich, 1987). Cada cadeia pesada pode ser separada em duas porções: meromiosina leve, em forma de bastão, e meromiosina pesada, conhecida como porção globosa da miosina, a qual apresenta o sítio de ligação com a actina e a região capaz de ligar-se à molécula de ATP e hidrolisá-la (atividade ATPásica) (Huxley 1969; Lowey et al. 1969) (Figura 3).

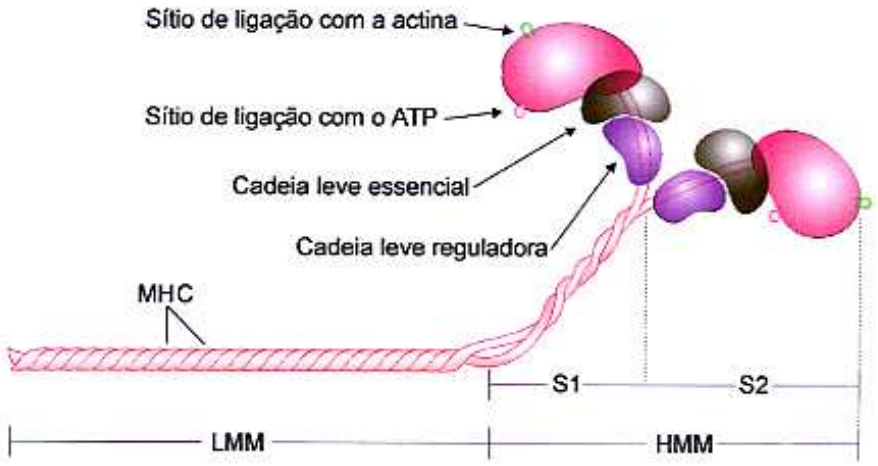

Figura 3. Esquema da molécula de miosina da classe II. Cada molécula de miosina é composta por duas cadeias pesadas de miosina (MHC) e quatro cadeias leves de miosina (MLC). As MHC podem ser clivadas e gerar as meromiosina leves (LMM) e meromiosina pesadas (HMM). As HMM são compostas pela porção α hélice em forma de bastão S1 e pela porção globosa S2. As MLC estão dispostas na proporção de duas cadeias (uma essencial e uma reguladora) para cada subfragmento S1 (Dal Pai-Silva et al., 2005).

Ashmore & Doerr (1971), utilizando a combinação das reações histoquímicas para detecção da atividade das enzimas m-ATPase e succinato desidrogenase (SDH), classificaram as fibras musculares como βRed, αRed e αWhite. Posteriormente, Peter et al., (1972), classificaram as fibras musculares em SO (slow oxidative), FOG (Fast oxidative glycolytic) e FG (Fast glycolytic), baseando-se na combinação das reações

histoquímicas e na detecção da atividade das enzimas m-ATPase e NADH tetrazólio redutase (NADH-TR).

Estudos mais recentes, envolvendo a microdissecção de fibras e, associando a reação histoquímica m-ATPase com a técnica da eletroforese, possibilitaram a separação de quatro isoformas de cadeia pesada de miosina (MHC) presentes nas fibras musculares: fibras do tipo I, com MHCI, fibras do tipo IIA, com MHC IIa, fibras do tipo IIB, com MHC IIb e fibras do tipo IID com MHC IId (Termin et al. 1989). A MHC IId está presente nos músculos de pequenos mamíferos e possui uma velocidade de contração intermediaria entre as MHCIIa e MHCIIb (Hilber et al., 1999). As fibras IID apresentam características histoquímicas e bioquímicas similares às fibras 2X descritas em ratos (Larsson et al., 1991), camundongos e coelhos (Hämäläinen & Pette, 1993), sendo também denominadas de fibras IID/IIX (para uma revisão ver Scott et al., 2001). Baseado em vários tipos de evidências e na análise de seqüências de DNA, a MHC originalmente identificada em humanos como MHCIIb é na verdade homóloga à MHCIId/IIx presente nas fibras IID/IIX de pequenos mamíferos (Pette & Staron, 1997). Portanto, os humanos expressam as seguintes isoformas de MHC (da mais lenta para a mais rápida): MHCI, MHCIIa e MHCIIx/d (Staron, 1997); e não expressam a mais rápida isoforma de todas as MHC, a MHCIIb (Hilber et al., 1999).

As fibras do tipo I, IIA, IID/X e IIB são classificadas como fibras puras (Pette & Staron, 1997; Staron et al., 1999). Porém, além das fibras puras, que expressam apenas um tipo de RNA mensageiro para a MHC, há fibras que co-expressam diferentes genes para a MHC (Biral et al., 1988; Aigner et al, 1993; Schiaffino & Reggiani, 1994; Caiozzo et al., 2003). Essas fibras são classificadas de acordo com o tipo de MHC predominante: (IC=MHCI>MHCIIa, IIC=MHCIIa>MHCI, IIAD=MHCIIa>MHCIId, IIBD=MHCIIb>MHCIId), sendo denominadas de fibras híbridas ou polimórficas (Staron & Pette, 1993; Di Maso et al., 2000).

A velocidade de contração de uma fibra muscular está diretamente relacionada com o tipo de MHC (revisado em Talmadge et al., 1993). A MHC capaz de rápida hidrólise do ATP é característica das fibras do tipo II, que são fibras de contração rápida. Já a MHC de baixa atividade ATPásica é encontrada nas fibras do tipo I, de contração lenta (Kelly & Rubinstein, 1994).

A identificação das características contráteis das fibras musculares é importante, pois como os músculos são compostos por vários tipos de fibras musculares, suas propriedades refletem a soma das características das fibras que o constituem.

Vários estudos procuraram investigar as possíveis correlações entre as diferentes isoformas de miosinas e as propriedades metabólicas oxidativas e glicolíticas das fibras musculares (Pette & Staron, 2001). Desta forma, a combinação entre o padrão de reação para a atividade da mATPase e reações histoquímicas de algumas enzimas metabólicas, foram utilizadas para classificar as fibras musculares de acordo com o seu metabolismo energético (Pette & Staron, 1997).

Rivero et al., (1999), utilizando-se de métodos histoquímicos, investigaram as interrelações entre a atividade da mATPase, a atividade das enzimas metabólicas (succinato desidrogenase, SDH e α-glicerolfosfatase desidrogenase, GPD) e a área de secção transversal das fibras musculares do músculo gastrocnêmio de ratos. Este estudo indicou uma correlação positiva entre a isoforma de MHC e atividades das enzimas mATPase e GPD (enzimas associadas ao metabolismo glicolítico), na qual evidenciou um padrão de atividade destas enzimas, de acordo com o tipo de fibra: IIB>IID/XB>IID/X>IIAX>IIA>I+IIA>I. Por outro lado, a atividade da SDH, enzima associada ao metabolismo oxidativo, foi maior nas fibras I>IIA>IIB. Contudo, as fibras com maior Area Sseccional Transversa- AST (IIB e IID/X), apresentaram maior atividade da GPD e menor atividade da SDH, inversamente, as fibras com menor AST (I e IIA), apresentaram maior atividade da SDH e menor atividade da GPD. Estes resultados apontam uma associação entre a isoforma de miosina expressa, AST e propriedade metabólica muscular, de acordo com as características contráteis, morfológicas e bioquímicas, a fim de garantir a especificidade funcional dos diferentes músculos.

De acordo com os vários parâmetros descritos para identificar os diferentes tipos de fibras musculares, tais como: as diferenças nas isoformas das MHCs, o perfil das enzimas metabólicas, as características bioquímicas e fisiológicas, e suas propriedades estruturais e contráteis (Dubowitz & Pearse, 1960; Pette & Staron, 2001; D' Antona et al., 2006), tem sido utilizada uma nomenclatura geral para classificar os diferentes tipos de fibras musculares: Fibras de contração lenta – Tipo I (slow-twitch fibers),

dependentes do metabolismo oxidativo (SO – slow oxidative); Fibras de contração rápida – Tipo IIA (fast-twitch fibers), dependentes do metabolismo oxidativo e glicolítico (FOG – fast oxidative and glycolytic) e Fibras de contração rápida - Tipo IIB (fast-twitch fibers), dependentes do metabolismo glicolítico (FG – fast-twitch glycolytic) (Peter et al., 1972; Simoneau & Bouchard, 1995).

3.3. Plasticidade do Músculo Esquelético

O músculo esquelético possui uma alta plasticidade, podendo alterar suas características morfológicas, metabólicas, contráteis e funcionais de suas fibras musculares em diversas situações como em estados patológicos e exercício físico. A insuficiência cardíaca é uma dessas condições patológicas que induz adaptações qualitativas e quantitativas nas propriedades do músculo esquelético.

3.4. Disfunção Cardíaca e Insuficiência Cardíaca

A disfunção cardíaca precede a Insuficiência Cardíaca (IC). A disfunção cardíaca é caracterizada por anormalidades do relaxamento e/ou contração cardíaca sem apresentar retenção hídrica e intolerância ao esforço (Opie 2004).

A IC é um estado fisiopatológico no qual o coração é incapaz de bombear sangue de acordo com as necessidades metabólicas teciduais, ou pode fazê-lo adequadamente à custa da elevação da pressão de enchimento ventricular (Braunwald et al. 2001). De acordo com Cohn (1988), a IC é uma síndrome clínica associada à disfunção cardíaca, diminuição da expectativa de vida e intolerância aos exercícios físicos.

As principais causas da IC são isquemias, inflamações agudas, hipertensão arterial e alterações das valvas cardíacas (Francis 2001).

A IC constitui um importante problema clínico devido à gravidade de suas manifestações e à sua grande prevalência. Dados obtidos nos Estados Unidos e na Europa mostram que a incidência média de IC é de 1 a 5 casos por 1000 habitantes/ano, e sua prevalência é de aproximadamente 1% a 2% da população

(Cowie et al., 1997). No Brasil não existem estudos epidemiológicos envolvendo a incidência de insuficiência cardíaca. Porém, de acordo com outros países pode-se estimar que até 6,4 milhões de brasileiros sofram de insuficiência cardíaca (Guimarães et al., 2002). Conforme dados publicados pelo Ministério da Saúde, foram realizadas nos primeiros sete meses de 2003, 203.893 internações por insuficiência cardíaca, com ocorrência de 14 mil óbitos e taxa de mortalidade de 14,7. A IC encontra-se entre as principais causas de internação do Sistema Único de Saúde (Albanesi Filho, 1998; Rossi Neto, 2004).

Entre os principais sintomas da IC encontram-se: dispnéia, fraqueza e fadiga de membros inferiores com conseqüente redução da atividade locomotora, intolerância para realizar exercícios físicos e piora da qualidade de vida (Poole-Wilson & Ferrari, 1996; Wilson, 1996; Bigard et al., 1998). Importantes alterações ocorrem na morfologia e função cardíaca. Porém, estudos demonstraram pobre correlação entre débito cardíaco, fluxo sanguíneo e intolerância ao exercício, sugerindo como principais contribuintes para a incapacidade funcional, as alterações periféricas musculares (Vescovo et al., 1998; De Sousa et al., 2002).

3.5. Remodelação Cardíaca na Insuficiência Cardíaca

Em resposta à sobrecarga hemodinâmica provocada por alterações isquêmicas, hipertensivas, valvares e outras, ocorre um mecanismo adaptativo que permite ao coração manter suas funções em vigência de aumento de carga, processo denominado remodelação cardíaca (RC) (Cicogna et al., 2000; Olivetti et al., 2000).

A RC é um processo adaptativo, tempo-dependente, resultante de sobrecarga hemodinâmica crônica, caracterizada por alterações moleculares, estruturais e funcionais (Okoshi et al., 2004; Opie et al., 2006). Na RC ocorre mudanças moleculares, celulares e intersticiais miocárdicas, que se expressa por variação no tamanho, forma e função cardíaca (Cohn et al, 2000).

Entre as adaptações estruturais que ocorrem na RC destacam-se a hipertrofia do miócito e da célula muscular lisa vascular e alterações na matriz extracelular. É considerado um processo compensatório sendo, entretanto, preditor de eventos

cardiovasculares como, isquemia miocárdica, insuficiência cardíaca, arritmias e morte súbita (Wettschureck et al., 2001; Swynghedauw, 2006).

Diferentes modelos experimentais têm sido propostos para o estudo da RC por sobrecarga pressórica como a estenose da artéria renal (Okoshi et al., 1997), da aorta abdominal (Rossi & Peres, 1992; Rodrigues et al., 1992) e nos ratos espontaneamente hipertensos (SHR) (Bing et al., 1995).

O modelo de estenose da aorta supravalvar (EAo) tem sido amplamente utilizado para o estudo remodelação ventricular, sendo que este modelo assemelha-se parcialmente à EAo que ocorre em humanos (De Sousa et al., 2002; Boluyt et al, 2005; Bregagnollo et al, 2006 e 2007). A estenose aórtica supravalvar tem como principal causa em adultos a calcificação, muito semelhante a aterosclerose (Bonow et al., 2006).

As vantagens da EAo supravalvar são o desenvolvimento gradual de hipertrofia ventricular esquerda, ausência de severas lesões anatômicas no miocárdio e reduzido custo de manutenção devido ao curto período (quando comparado ao modelo SHR) necessário para o desenvolvimento da remodelação e insuficiência cardíaca. A EAo acarreta hipertrofia ventricular concêntrica evidente após duas semanas do processo cirúrgico e mantém-se estável até 12 semanas (Ribeiro et al., 2004). A função cardíaca, dependendo do período, pode estar normal, aumentada ou deprimida (Ribeiro et al., 2004, Boluyt et al., 2005, Bregagnollo et al., 2006). A transição entre disfunção ventricular e insuficiência cardíaca ocorre aproximadamente a partir de 18-20 semanas (Feldman et al., 1993; Weinberg et al., 1994; Ribeiro et al., 2003).

Na RC ocorrem várias alterações na morfologia dos cardiomiócitos como hipertrofia, desorganização das miofibrilas, fibrose intersticial, apoptose e necrose; alterações no metabolismo energético, no acoplamento contração-excitação, distúrbios do Ca^{+} intracelular e re-expressão de genes fetais (Cohn et al., 2000). Outros componentes cardíacos também são afetados como o sistema arterial coronariano (disfunção endotelial, hiperplasia de musculatura lisa, rarefação capilar) (Cohn et al., 2000). Ocorre um aumento gradual dos diâmetros sistólico e diastólico do ventrículo esquerdo, mudanças para um padrão mais esférico da câmara ventricular associado a um declínio da fração de ejeção do ventrículo esquerdo. Os resultados destes

processos incluem piora progressiva das funções sistólicas e diastólicas, desenvolvimento de regurgitação mitral e aumento dos riscos de arritmias (Pieske, 2004).

A RC é associada com piora do prognóstico na insuficiência cardíaca e a sua prevenção é considerada alvo terapêutico (Pieske 2004).

3.6. Alterações nas fibras do Músculo Esquelético na Insuficiência Cardíaca e possíveis mecanismos envolvidos

Embora vários fatores tenham sido descritos como responsáveis pelo desenvolvimento de fadiga nos pacientes com IC, sua etiopatogenia ainda não está completamente esclarecida. Esse fenômeno é decorrente, em parte, das alterações metabólicas, com aumento do metabolismo glicolítico, decréscimo do metabolismo oxidativo e menor resistência à fadiga (Simonini et al.,1996; Lunde et. al., 200; Ventura-Clapier et al., 2003).

Na IC, observa-se também, a atrofia da musculatura esquelética, em aproximadamente 68% dos pacientes com essa síndrome (Mancini et al., 1992; Harrington et al., 1997; Poehman, 1999; De Sousa et al., 2000; Carvalho et al., 2003).

A IC induz a expressão da isoforma de cadeia pesada de miosina (MHC) em direção a isoforma rápida (Simonini et al. 1996; Bigard et al., 1998; Vescovo et al. 1998; Carvalho et al., 2003), a qual está relacionada com a severidade da IC (Vescovo et al., 1996; Spangenburg et al., 2002). Dados do nosso grupo de pesquisa demonstraram em ratos com IC induzida por estenose aórtica, que na fase de hipertrofia cardíaca (18 semanas) o músculo sóleo já apresenta mudança para um padrão fenotípico mais rápido (Carvalho et al., 2003).

É provável que os MRFs, MyoD, miogenina, Myf5 e o MRF4, tenham participação nas mudanças nos tipos de fibras. Como descrito anteriormente, na miogênese, esses fatores transcricionais músculo-específicos regulam a ativação, proliferação e diferenciação de células miogênicas. A MyoD e a Myf5 são expressos em mioblastos na fase de proliferação, que antecede a de diferenciação, enquanto que

a miogenina e o MRF4 são expressos em células no final da fase de diferenciação (Megeney & Rudnicki, 1995).

Na fibra muscular adulta, a miogenina e a MyoD também podem estar envolvidas na manutenção do seu fenótipo, rápido ou lento; a Miogenina é expressa em níveis superiores aos da MyoD em músculos lentos, enquanto que o oposto é verdadeiro para músculos rápidos (Hughes et al., 1993; Hughes et al., 1997; Voytik et al., 1993). Como na IC existe transição das isoformas de miosina de lenta para rápida, é provável que essa alteração seja decorrente de uma mudança na expressão dos fatores de regulação miogênica, MyoD e miogenina. No entanto, estudos têm evidenciado que a miogenina está mais relacionada com o metabolismo do músculo do que com as mudanças na composição das MHCs (Hughes et al., 1999; Siu et al. 2004).

Estudos têm demonstrado que as alterações na expressão dos MRFs estão diretamente envolvidas no controle fenotípico muscular e nas alterações metabólicas, em resposta a várias condições como alterações hormonais, microgravidade e o exercício físico (Mozdiziak et. al., 1998, Mozdiziak et. al., Hughes et. al., 1999). Entretanto há poucas informações na literatura a respeito do papel dos fatores de regulação miogênica na transição dos tipos de fibras musculares e das isoformas de cadeia pesada de miosina que ocorre nos portadores de disfunção cardíaca e insuficiência cardíaca.

Dados do nosso laboratório evidenciaram a participação dos MRFs na transição fenotípica do músculo diafragma de ratos em modelo de IC induzido por monocrotalina. Houve uma diminuição da expressão de MHC rápidas, associada a uma diminuição da MyoD; sem alterar a expressão da miogenina e do MRF4 (Lopes et al., 2007). Em outro estudo com o mesmo modelo experimental, porém, nos músculos dos membros mostramos a redução da MyoD nos músculos soleus e extensor longo dos dedos (EDL), enquanto que a migenina não alterou. Nenhuma modificação foi encontrada nas MHCs. Provavelmente essa alteração gênica precedeu as alterações fenotípicas musculares.

A causa da alteração da MyoD na IC é desconhecida. Entretanto, a ativação neuro-hormonal e o aumento de citocinas podem contribuir (Anker et al., 1999). Na IC as citocinas inflamatórias podem ser ativadas, dentre elas o TNF-α (Levine et al., 1990;

MucMurray et al., 1991, Dalla Libera et al., 2001). Este mediador inflamatório está relacionado com a perda de massa muscular e caquexia nesses pacientes (Levine et al., 1990). O TNF-α atua diminuindo o RNAm da MyoD a nível pós-transcricional (Israel 2000) e em cultura de células o TNF-α inibe a diferenciação miogênica através da desestabilização protéica da MyoD (Langen et al. 2004).

O TNF-α também está relacionado com o hormônio anabólico IGF-I (insulin-like growth factor) (Fan et al. 1995). A infusão de TNF-α provoca a diminuição do IGF-I no fígado e no músculo, enquanto que o pré-tratamento com anti-TNF-α previne completamente o decréscimo do IGF-I no músculo. Em humanos com IC, a diminuição local do IGF-I no músculo esquelético, está associada com aumento de TNF-α e diminuição da expressão gênica da MHC do tipo I (Toth et al. 2005). Logo, as alterações hormonais e das citocinas inflamatórias podem contribuir para as disfunções músculo esqueléticas na IC.

3.7. Treinamento Físico na Insuficiência Cardíaca

Embora a atividade física tenha sido evitada em pacientes com IC até a década de 1980, na última década, o treinamento de físico mostrou-se aumentar a tolerância ao esforço, qualidade de vida e reduzir as taxas de morbidade e mortalidade (Coats, et al, 1990; Belardinelli, et al, 1999; Cohen et al., 1999; Coats 2000; Piepoli et al., 2004; Pina et al., 2004).

3.8. Treinamento Fïsico e Remodelação Cardíaca

Medidas farmacológicas (Khattar et al., 2001; Doughty et al., 2004) e não farmacológicas como o exercício físico (Kavanagh et al 2002; Giannuzzi et al., 2003; Wisloff et al., 2007) têm sido propostas para reverter ou amenizar as alterações da RC. Em estudos recentes tem sido crescente o consenso de que o exercício físico é benéfico para pacientes com doenças cardiovasculares mesmo naqueles com alterações severas da função cardíaca, e a inatividade física acelera a severidade da insuficiência cardíaca (Kavanagh et al., 2002; Wisloff et al., 2007). Entretanto se o

treinamento físico produz qualquer efeito no desenvolvimento da IC esse fato é menos estudado.

O exercício físico é recomendado para indivíduos com estenose aórtica após avaliação clínica e ecocardiográfica (Bonow et al., 2005). Porém não foram encontrados estudos que avaliaram os efeitos do exercício físico na RC induzida por estenose aórtica.

Enquanto que a sobrecarga hemodinâmica pressórica, como na estenose da valva aórtica, induz hipertrofia patológica ou mal adaptativa, caracterizada por deterioração funcional e estrutural, o exercício físico crônico promove remodelamento cardíaco benéfico ou adaptativo, não associado à disfunção cardíaca e aumento de morbidade (Shapiro 1984; Strom et al., 2005). Entre as adaptações induzidas pelo treinamento físico, observa-se redução da freqüência cardíaca em repouso, aumento da função cardíaca e diminuição da freqüência cardíaca submáxima durante o exercício (Iemitsu et al., 2005).

São poucos os estudos que avaliaram a associação do treinamento físico e remodelação cardíaca patológica. Foi demonstrado que o treinamento físico por longo período (6 meses) e intensidade moderada, induz a reversão da remodelação cardíaca ocasionado pelo processo patológico (remodelamento reverso) em pacientes com IC estável. Foram constatadas melhora da fração de ejeção, diminuição do volume diastólico final e do volume sistólico final do ventrículo esquerdo. Esta melhora foi associada com aumento da capacidade funcional e consumo máximo de oxigênio pelos tecidos (Giannuzzi P et al., 2003).

Pacientes infartados com IC foram divididos em dois grupos, submetidos a 2 protocolos de treinamento por 12 semanas, 3 vezes por semana. Um dos grupos realizou exercício aeróbio contínuo moderado e o outro treinamento aeróbio intervalado. Estes autores demonstraram que o treinamento aeróbio intervalado melhorou a capacidade aeróbia e promoveu a remodelação reversa do ventrículo esquerdo, mais do que no outro protocolo. A diminuição do volume sistólico final, volume diastólico avaliado pelo ecocardiograma ocorreu apenas no treinamento intervalado (Wisloff et al., 2007).

Comparando a influência de dois tipos de treinamento (aeróbio e de força) e a combinação entre estes sobre a remodelação do ventrículo esquerdo, ficou comprovado que o treino aeróbio foi capaz de promover a remodelação reversa (aumento da fração de ejeção e diminuição do volume diastótico final) em pacientes com IC estável (Haykowsky et al., 2007).

3.9. Adaptações das Fibras Musculares ao Treinamento

As respostas aos diferentes modelos de treinamento aeróbico têm sido associadas a adaptações morfológicas e metabólicas dos músculos, como o aumento no número de mitocôndrias e na atividade das enzimas do metabolismo oxidativo, a elevação na concentração de proteínas mitocondriais (Stone et al., 1996; Hawley, 2002), e a melhora na captação de oxigênio em exercício submáximo (Demirel et al., 1999; Trappe et al., 2006).

As adaptações musculares agudas e crônicas, que ocorrem em resposta à relação estímulo/resposta de treinamento aeróbico, as quais promovem um aumento da capacidade oxidativa e antioxidante muscular, estão bem estabelecidas (Dudley, 1982; Powers, 1994). No entanto, as possíveis mudanças no perfil fenotípico das fibras musculares em resposta ao treinamento aeróbico, relacionadas ao padrão de recrutamento das fibras musculares, permanecem pouco esclarecidas.

Vários estudos procuram investigar as possíveis adaptações das fibras musculares a padrões de impulsos nervosos de baixa freqüência, assim como em modelos de treinamento físico de longa duração (treinamento de resistência ou aeróbico, endurance training) (Demirel et al., 1999; O'Neill et al., 1999; Trappe et al., 2006) e Estimulação Elétrica Crônica de Baixa Frequência (CLFS - Chronic low-frequency stimulation) (Salmons & Vrbova´, 1969; Simoneau & Pette, 1988, Putman et al., 2004a). A CLFS causa maiores mudanças no fenótipo das fibras musculares comparada ao treinamento aeróbico, as quais seguem uma sequência de ajuste, das isoformas rápidas em direção as isoformas lentas, como descrito em músculos de contração rápida de ratos (MHCIIb → MHCIId → MHCIIa) e coelhos (MHCIId → MHCIIa → MHCI) (Pette & Staron, 2000). As adaptações fenotípicas das fibras

musculares, aos estímulos da CLFS são quantitativamente maiores, mas qualitativamente similares quando comparadas ao estímulo pelo treinamento físico (Pette & Staron, 2001).

Embora os estímulos dos diferentes protocolos de treinamento aeróbico sejam suficientes para provocar um ajuste das fibras rápidas em direção a lentas (IIB → IIA) (Sullivan et al., 1995; Putman et al., 2004b), estas mudanças não atingem a transição entre os diferentes tipos de fibras, como observado na CLFS (IID → IIA → I) (Pette & Staron, 2001).

Em humanos, algumas evidências da modulação das fibras musculares, atingindo a transição de fibras rápidas para lentas (tipo II → tipo I), foram observadas em indivíduos que praticavam treinamento aeróbico há 10 anos. A análise do músculo vasto lateral revelou um maior percentual de fibras do tipo I no grupo treinado (70,9%), comparado ao grupo sedentário (37,7%), enquanto que o percentual de fibras do tipo II foi menor (25,3%) versus (51,8%), no sedentário (Thayer et al., 2000). Em adição, Harber et al., (2002), observaram que o músculo gastrocnêmico de corredores de longa distância (fundistas), apresentava maior proporção de fibras do tipo I (MHCI) (71%), quando comparados a corredores de média distância (56,3%) e corredores recreativos (59,8%). Frente aos resultados, os autores sugerem um aumento na expressão de MHCI, posteriormente ao treinamento de corrida de longa distância, e uma prevalência de MHCIIa, após treinamento para eventos de média distância.

Contudo, embora alguns trabalhos apontem um aumento no percentual de fibras do tipo I (MHCI) seguida de treinamento aeróbico, existem evidencias limitadas da ocorrência de transição das fibras do tipo II para fibras do tipo I, independente do tipo de treinamento (tabela 1).

Tabela 1 – Representação esquemática da direção dos ajustes das fibras musculares ao treinamento aeróbico em humanos e animais. Presença de modulação (seta contínua), Ausência de modulação (seta interceptada), Possibilidade de modulação (seta descontínua).

	TREINAMENTO AERÓBICO
Humanos	Tipo I ← - - - - - - - - - - - - - - ← Tipo IIA ← - - - - - - - - - - - - - - ← Tipo IIB
Animais	Tipo I ← - - - - - - - - - - - - - - ← Tipo IIA ← - - - - - - - - - - - - - - ← Tipo IIB

4.0 Mecanismos envolvidos nas alterações musculares com o Treinamento Físico Aeróbio

As adaptações fenotípicas musculares observadas em diferentes modelos de treinamento físico são dependentes da força, velocidade e duração dos padrões de contração muscular (Impulso nervoso), cuja magnitude está associada aos estímulos extrínsecos (carga ou estresse mecânico) e intrínsecos (níveis de cálcio intracelular e hipóxia) (Baar et al., 1999).

Os estímulos específicos (perturbações mecânicas, estiramento, microlesão/injúria e estresse celular), originados de diferentes tipos e protocolos de exercício físico são transduzidos por receptores de superfície celular (moléculas transmembranas), ativando uma "cascata" de moléculas intracelulares (vias moleculares), que integram esta informação (Wackerhage & Woods, 2002), e assim, controlam as mudanças quantitativas e qualitativas no músculo, por meio da ativação ou repressão de genes músculo específicos (Bassel-Duby & Olson, 2006). Pesquisas recentes apontam à participação de várias vias moleculares no controle do fenótipo muscular, incluindo a via do IGF-I (insulin-like growth factor, fator de crescimento ligado à insulina) (Tidball, 2005).

Vários estudos fornecem evidencias da atuação do IGF-I como um potente sinal anabólico no tecido muscular (Glass et al., 2003; Goldspink, 2005). Os sinais mecânicos que atingem as células musculares, como por exemplo, a perturbação mecânica nas fibras musculares, ocasionada pelo processo de contração durante o

exercício físico, induzem à liberação do IGF, que se liga ao receptor na superfície celular, e, assim, ativa uma “cascata” de eventos intracelulares e a síntese protéica.

Diferentes vias de sinalização intracelular são ativadas de acordo com a especificidade das respostas funcionais, na qual múltiplos processos são necessários para regular a expressão de genes específicos, responsáveis pelas alterações das propriedades contráteis e metabólicas das fibras musculares.

O exercício físico regula as propriedades contráteis e metabólicas do músculo esquelético, e alterações na expressão gênica dos MRFs MyoD e Miogenina contribuem para as alterações musculares. Psilander et al. (2003) demonstraram no músculo vasto lateral de humanos, que uma única série de exercício de resistência aumenta a expressão da MyoD e miogenina, o que suporta a hipótese de que os MRFs estão envolvidos no mecanismo de hipertrofia e transição fenotípica. Hughes et al. (1999) demonstraram em animais transgênicos, a atuação da miogenina na transição do metabolismo de glicolítico para oxidativo, sem alterar as MHCs. Siu et al. (2004) demonstraram no músculo sóleo de ratos submetidos a um programa de exercício aeróbico por 8 semanas, que a miogenina está linearmente relacionada com adaptações das enzimas do metabolismo oxidativo, porém não houve alteração da MyoD e do perfil contrátil.

4.1. Treinamento Físico no músculo esquelético na Insuficiência Cardíaca

Na IC, o exercício físico é uma conduta proposta e amplamente aceita para minimizar as conseqüências dos sintomas causados por essa patologia (Pinã, et al., 2003). Com exercício físico regular, há melhora da tolerância ao esforço, na capacidade funcional e na qualidade de vida dos pacientes, melhorando o metabolismo oxidativo e o padrão contrátil dos músculos (Taylor, 2000; De Sousa et al., 2002; Pinã et al., 2003).

Em pacientes com insuficiência cardíaca crônica um programa de seis meses de exercício aeróbico, remodelou as fibras do músculo gastrocnêmio para o tipo I, revertendo à mudança causada pela IC (Hambrecht et al., 1997). De Sousa et al. (2002) observaram um aumento da MHC IIa, decréscimo da capacidade oxidativa e

alteração na função mitocondrial, no músculo sóleo de ratos com IC induzida por estenose aórtica. Após 8 semanas de exercícios voluntários em esteira, houve modificação fenotípica do músculo para um padrão mais lento e perfil oxidativo, com aumento das enzimas creatina kinase (CK) e citrato sintase (CS). Em humanos foi demonstrado que as anormalidades metabólicas e funcionais dos músculos periféricos (membros superiores) são melhoradas diretamente por exercício físico, sem alterar a performance cardíaca (Minotti et al., 1990).

O exercício físico na IC também promove elevação muscular do IGF-I, o que indica que esse tipo de intervenção reverte parcialmente o estado catabólico no músculo esquelético (Hambrecht et al., 2000 e 2005). Nesta condição ocorre redução dos níveis de TNF-α, o que confirma os efeitos benéficos anti-inflamatórios musculares na IC (Gielen et al., 2003). Em pacientes com IC, houve diminuição local da expressão do TNF-α no músculo quadríceps, após exercícios de endurance (Hambrecht et al., 1999).

A hipótese deste estudo é que a IC pode alterar os MRFs e que a aplicação do TF antes de se instalar a IC promoverá melhora na RC e reverterá às alterações fenotípicas (Fast-Slow) do músculo esquelético, nos MRFs MyoD e mogenina e seus possíveis mecanismos de controle que seriam o TNF-α/IGF-I e citrato sintase, respectivamente.

Objetivos

Avaliar a influência do treinamento físico em ratos Wistar, na transição entre disfunção ventricular e insuficiência cardíaca induzida pela estenose aórtica:

1. Na remodelação cardíaca;
2. Nas características morfológicas e metabólicas; na expressão dos Fatores de Regulação Miogênicos (MyoD e Miogenina); na expressão do IGF-I e TNF-α, no músculo estriado esquelético;

ARTIGO 1

PHYSICAL TRAINING DELAYS THE TRANSITION FROM LEFT VENTRICULAR DYSFUNCTION TO HEART FAILURE IN RATS WITH AORTIC STENOSIS

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ABSTRACT

Background: Aortic stenosis (AS) is used for the study of cardiac remodeling (CR) by pressure overload. The physical training (PT) is a proposal applied in heart failure (HF). The purpose of this study was to determine whether PT may alter the CR in rats with AS. **Methods and Results:** There were 6 groups: aortic stenosis 18 weeks (AS18), Control 18 weeks (C18), aortic stenosis 28 weeks training (ASTR), aortic stenosis 28 weeks control (AS28), Control 28 weeks (C28) and Control 28 weeks training (TR). After 18 weeks of AS, when the animals presented ventricular dysfunction, they were submitted to PT during 10 weeks. HF was evaluated by clinical data and CR by morphologic data and echocardiogram. AS28 showed clinical signs of HF, ASTR presented decrease of them. Atrium and right ventricle/body weight relations, the systolic and diastolic diameters, left atrium Diameter/Ao Diameter relations and waves E/A mitral decreased; the endocardic shortening percentage, speed of posterior wall shortening of the left ventricle increased in ASTR. **Conclusions:** PT improved the ventricular function and it decreased the clinical signs of CR.

Keywords: aortic stenosis, cardiac remodeling, echocardiography, physical training, rats

INTRODUCTION

Heart Failure (HF) is the main cause of hospitalization and death in the world [1]. Cardiac dysfunction that precedes HF is characterized by abnormalities of cardiac relaxation and/or contraction without water retention or exercise intolerance [2]. The main causal events of HF are ischemia, acute inflammations, arterial hypertension and valve alterations [3,4].

In response to hemodynamic overload provoked by these causes, an adaptive mechanism occurs that permits the heart to maintain its functions in terms of increased load, a process denominated cardiac remodeling (CR) [5,6]. CR is an alteration in gene expression in response to an aggression, resulting in molecular, cellular and interstitial myocardial changes that are expressed by variation in cardiac size, form and function [7]. To analyze the effects of CR various experimental models have been utilized including supravalvar aortic stenosis (AS), which partially resembles AS in humans [8-10].

Both pharmacological [11-12] and non-pharmacological measures [13-14] have been proposed to reverse or mitigate CR alterations. In recent studies there has been a growing consensus that physical training is beneficial for patients with cardiovascular diseases, even those with severe alterations of cardiac function [13-14].

Physical training is indicated for patients with AS after clinical and echocardiographic evaluation [15]. However, we found no studies that evaluate the effects of PT on CR induced by AS in humans or an experimental model.

The present work aimed to test the hypothesis that physical training delays the transition from ventricular dysfunction to heart failure in rats with AS by attenuating heart remodeling.

MATERIALS AND METHODS

Experimental animals and study protocol

All experiments and procedures conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication no. 85-23, revised 1996; http://www.nap.edu/openbook. php?record_id=5140) and were approved by the Animal Ethics Committee (Sao Paulo State University, UNESP). Male Wistar weaning rats (3–4 weeks old), weighing 90–100 g, were anaesthetized with a mixture of ketamine (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Aortic constriction was created by placing a 0.6 mm i.d. stainless-steel clip on the ascending aorta via a thoracic incision, as previously described [16,17] Control animals underwent left thoracotomy without clip placement (n = 24). All rats were housed in a temperature-controlled room (23±C) on an inverted 12 h light–dark cycle, with food and water supplied *ad libitum*. Eighteen weeks after surgery, part of Control animals (C18, n=4) and part of aortic stenosis animals (AS18, n=4) were sacrificed. Another part of Control and AS animals were divided in 4 groups: aortic stenosis 28 weeks training (ASTR, n=8), aortic stenosis 28 weeks control (AS28, n=6), Control 28 weeks (C28, n=9) and Control 28 weeks training (TR, n=6).

Physical training protocol

The training protocol utilized was modified from De Souza et al., (2002) [18] and Siu PM, et al*,* (2004) [19]. The animals in the ASTR group were submitted to a treadmill training program, five times per week, for ten weeks. The training protocol is described in Table 1.

Table 1. Physical training protocol

Weeks	Velocity (m/min)	Duration (min)
1	5	10
2	7,5	12
3	10	14
4	10	16
5	10	18
6	10	20
7	10	20
8	10	20
9	10	20
10	10	20

Echocardiography

Rats were anaesthetized with a mixture of ketamine (50 mg/kg, i.m.) and xylazine (1 mg/kg, i.m.). The chest was shaved and rats were positioned on their left side. Using an echocardiograph (HDI 5000 SonoCT; Philips) equipped with a 12 MHz transducer, a two-dimension guided M-mode images were obtained. M-Mode tracings

were obtained from long-axis views of the LV at or just below the tip of the mitral valve leaflets and at the level of the aortic valve and left atrium [20-22]. M-Mode images of the LV were recorded on a black-and-white thermal printer (UP-890 MD; Sony, Tokyo, Japan) at a sweep speed of 100 mm/s. All LV tracings were measured manually by the same observer, who was blinded to the treatment group, according to the leading-edge method of the American Society of Echocardiography [23]. Measurements were the mean of at least five cardiac cycles on the M-mode tracings. The following variables were measured: heart rate (HR), LV diastolic dimension (LVDD), LV systolic dimension (LVSD), LV posterior wall thickness (LVWT), interventricular septum thickness in diastole (IVS), LV relative thickness in diastole (LVRT), endocardial fractional shortening, posterior wall shortening velocity (PWSV).

LV diastolic dimension (LVDD) and posterior wall thickness (LVWT) were measured at maximal diastolic dimension, and LV systolic dimension (LVSD) was taken at maximal anterior motion of posterior wall.

The LV systolic function was assessed calculating the fractional shortening (FS) = {(LVDD–LVSD)/LVDD×100}. Posterior wall shortening velocity, PWSV, which is the velocity corresponding to the maximum tangent of the systolic movement of the posterior wall.

The study of LV diastolic function measured peaks of transvalvar mitral flow velocities corresponding to the initial filling phase (wave E) and the late phase corresponding to atrial contraction (wave A), as well as the wave E/wave A ratio. E/A ratio was used as an index of LV diastolic function. Left atrium (LA) was measured at its maximal diameter and aorta (Ao) at end of diastole LA/Ao.

The echocardiogram was accomplished 18 and 28 weeks after AS induction in all animals of the experimental groups. In the 28-week group the evaluation was performed three days after the finalization of training.

Anatomical parameters

At the end of the 18^{th} and 28^{th} weeks, the animals were anesthetized intraperitonially with sodium pentobarbital, 50 mg/kg and sacrificed.

The anatomical variables utilized to characterize CR were final body weight (FBW) and weights of LV, RV and atria (ATs), and the ratios LV/FBW, RV/FBW and ATs/FBW (Tables 2 and 5).

Clinical sign of HF

The presence of heart failure was evaluated, by tachypnea, and at rat sacrifice, by the presentation of pleural effusion, ascites, left atrium thrombi and hypertrophy of the right ventricle [9,10,24,25].

Statistical analysis

The anatomical and echocardiogram parameters in the C18 and AS18 groups were analyzed by the Student's t test for independent samples when the variable was shown to adhere to a normal probability distribution (data were expressed as mean ± standard deviation) and by the non-parametric test of Mann-Whitney (data were expressed as median ± total semi amplitud) [26] when this characteristic was absent.

The echocardiogram and anatomical parameters in the C28, TR, AS28 and ASTR were analysed by two way ANOVA variance, followed by Student Newman Keuls Method (data were expressed as mean ± standard deviation).

In all tests, the significance level was set at 5% ($p<0.05$). Statistical calculations were accomplished with the aid of the statistical software package *SigmaStat* 3.5 *for Windows version* (*Copyright*© 2006, *Systat Software Inc*.).

RESULTS

After 18 weeks of AS induction no anatomo-clinical signs of HF were observed such as tachypnea, ascites, pleural effusion, thrombus in the left atrium or RV hypertrophy. However, the animals presented evidence of CR demonstrated by elevations of anatomical parameters, namely ATs/FBW, LV/FBW and Left Ventricle (LV) (Table 2).

Table 2. Anatomical parameters from aortic stenosis 18 weeks (AS18, *n*=4) and control 18 weeks (C18, *n*=4) groups.

Parameters	Group	
	C18	**AS18**
Body Weight (g)	404 ± 61	470 ± 45
LV (g)	0,82 ± 0,19	1,35 ± 0,13*
LV/FBW (mg/g)	2,01 ± 0,17	2,88 ± 0,12*
RV (g)	0,25 ± 0,06	0,29 ± 0,02
RV/FBW (mg/g)	0,61 ± 0,09	0,61 ± 0,04
ATs (g)	0,11 ± 0,01	0,20 ± 0,05*
ATs/FBW (mg/g)	0,28 ± 0,01	0,42 ± 0,10*

FBW: final body weight; LV: left ventricular weight; RV: right ventricular weight; ATs: atrium weight. Values are means ± SD. *p<0,05 compared to C18.

Echocardiographic evaluation confirmed the anatomical data such as increase in diastolic thickness of the posterior wall (LVWT) and of the interventricular septum (ISV) and the LA/Ao ratio. The observation of diastolic dysfunction was demonstrated by elevation of the mitral E/A wave ratio. Although there was no alteration in the LV endocardial fractional shortening, the posterior wall shortening velocity was lower, which may signify an alteration in systolic function (Table 3).

Table 3. Echocardiographic data from aortic stenosis 18 weeks (AS18, *n*=18) and control 18 weeks (C18, *n*=19) groups.

Parameters	Group	
	C18	**AS18**
Heart rate (bpm)	269 ± 21	295 ± 41
LVDD (mm)	8,44 ± 0,43	8,69 ± 1,2
LVSV (mm)	4,12 ± 0,52	4,24 ± 1,4
LVWT (mm)	1,55 ± 0,11	2,15 ± 0,35*
ISV (mm)	1,55 ± 0,09	2,15 ± 0,35*
Tickness Relative LV	0,36 ± 0,04	0,48 ± 0,10*
EFS (%)	51,31 ± 4,49	52,27 ± 9,91
PWSV (mm/s)	40,58 ± 6,16	29,80 ± 2,59*
E/A	1,66 ± 0,18	4,71 ± 3,24*
LA/Ao	1,36 ± 0,12	1,95 ± 0,43*

LVDD: left ventricular diastolic dimension, LVSD: ventricular systolic dimension, LVWT: left ventricular posterior wall thickness, ISV: interventricular septum thickness; EFS: endocardial fractional shortening; PWSV: posterior wall shortening velocity; E/A: E wave mitral flow; A wave mitral flow; LA/Ao: left atrium/aorta. Values are means ± SD. * $p<0,05$ compared to C18.

Animals of the 28-week AS group showed clinical signs of HF, whereas the ASTR group presented reduced intensity and frequency of these signs (Table 4).

Table 4. Clinical data from aortic stenosis 28 weeks (AS28, *n*=6) and aortic stenosis 28 weeks with physical training (ASTR, *n*=8) groups.

HF signs	Groups	Relative frequency (%)
Tachypnea	AS28	100%
	ASTR	12,5%
Ascites	AS28	66%
	ASTR	25,5%
	ASTR	12,5%
Pleural Effusion	AS28	33%
	ASTR	33%
	AS28	50%
	AS28	16%
	ASTR	12,5%
Left atrium thrombi	ASTR	25%
	AS28	17%
	AS28	33%

After the training period, the ASTR group presented diminished anatomical parameters, namely the Right Ventricle/FBW (RV/FBW) and ATs/FBW ratios; there was no structural alteration in the LV (Table 5).

Table 5. Anatomical Parameters from control 28 weeks (C28, n=9), trained 28 weeks (TR, n=6), aortic stenosis 28 weeks (AS28, n=6) and aortic stenosis 28 weeks with physical training (ASTR, n=8) groups.

Parameters	Group			
	C28	**TR**	**AS28**	**ASTR**
Body Weight (g)	480 ± 44	436 ± 34	439 ± 59	426 ± 52
TLV (g)	0,81 ± 0,07	0,84 ± 0,11	1,38 ± 0,29*	1,25 ± 0,19**
LV/FBW (mg/g)	1,70 ± 0,10	1,88 ± 0,17	3,10 ± 0,33*	2,92 ± 0,21**
RV (g)	0,27 ± 0,04	0,24 ± 0,05	0,52 ± 0,13*	0,30 ± 0,05#
RV/FBW (mg/g)	0,57 ± 0,07	0,54 ± 0,05	1,10 ± 0,27*	0,70 ± 0,11**#
ATs (g)	0,10 ± 0,01	0,10 ± 0,00	0,32 ± 0,13*	0,20 ± 0,08**#
AT/FBW (mg/g)	0,21 ± 0,02	0,22 ± 0,04	0,72 ± 0,25*	0,45 ± 0,15**#

FBW: final body weight; TLV: total left ventricular weight; RV: right ventricular weight; ATs: atrium weight. Values are means ± SD. * p<0,05 compared to C28; ** p<0,05 compared to TR; # p<0,05 compared to AS28.

The echocardiographic evaluation in groups AS28 and ASTR showed elevation of heart rate that was not altered by exercise. The LVSD and LVDD (Figure 1) and the LA/Ao and mitral E/A waves ratio decreased in the ASTR group in relation to AS28. The

endocardial fractional shortening and posterior wall shortening velocity were higher in ASTR compared to AS28 (Table 6).

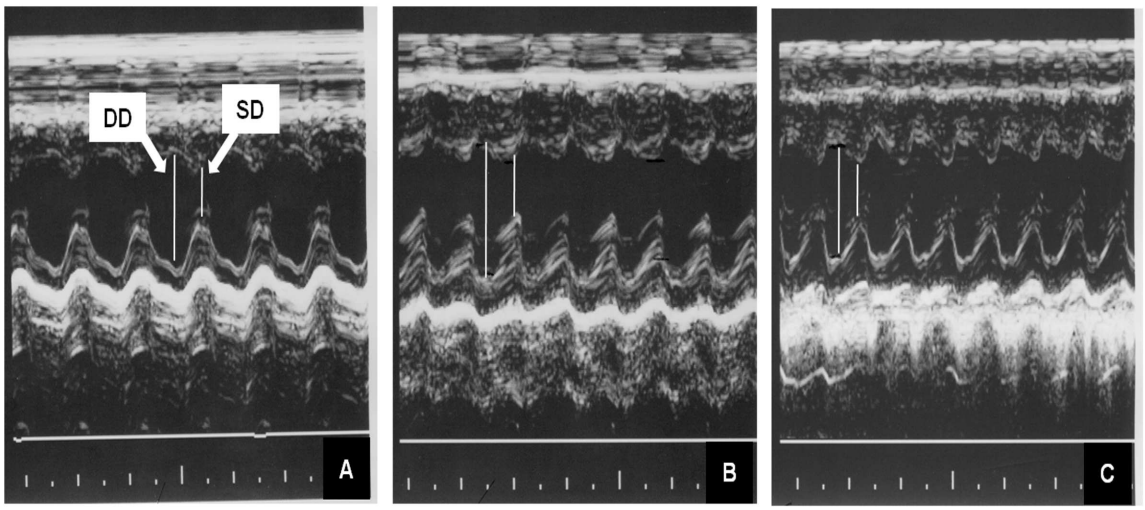

Fig. 1 Examples of M-mode echocardiograms of the left ventricle. SD: systolic diameter. DD: diastolic diameter. (A) control 28 weeks, (B) aortic stenosis 28 weeks (AS28) and (C) aortic stenosis training

Table 6. Anatomical Parameters from control 28 weeks (C28, n=9), trained 28 weeks (TR, n=6), aortic stenosis 28 weeks (AS28, n=6) and aortic stenosis 28 weeks with physical training (ASTR, n=8) groups.

Parameters	Groups			
	C28	TR	AS28	ASTR
Heart Rate (bpm)	265 ± 18	271 ± 18	319 ± 37*	320 ± 34**
LVDD (mm)	8,35 ± 0,58	8,03 ± 0,30	9,33 ± 1,30*	8,27 ± 1,03#
LVSD (mm)	4,11 ± 0,55	4,00 ± 0,47	5,70 ± 1,95*	4,03 ± 1,37#
LVWT (mm)	1,50 ± 0,17	1,51 ± 0,09	2,46 ± 0,37*	2,21 ± 0,23**
ISV (mm)	1,50 ± 0,16	1,53 ± 0,10	2,41 ± 0,26*	2,20 ± 0,22**
Tickness Relative LV	0,36 ± 0,04	0,40 ± 0,002	0,52 ± 0,10*	0,56 ± 0,06**
EFS (%)	50,79 ± 4,70	50,11 ± 4,70	40,90 ± 16,18	52,27 ± 11,60#
PWSV (mm/s)	38,63 ± 4,17	39,95 ± 4,84	24,31 ± 6,05*	30,96 ±3,94**#
E/A	1,66 ± 0,24	1,61 ± 0,20	5,20 ± 3,02*	2,86 ± 2,84#
LA/Ao	1,31±0,15	1,35±0,08	2,18±0,28*	1,88±0,26**#

LVDD: left ventricular diastolic dimension; LVSD: ventricular systolic dimension; LVWT: left ventricular posterior wall thickness; ISV: interventricular septum thickness; EFS: endocardial fractional shortening; PWSV: posterior wall shortening velocity; E/A: E wave mitral flow; A wave mitral flow; LA/Ao: left atrium/aorta. Values are means ± SD. * $p<0,05$ compared to C28; ** $p<0,05$ compared to TR; # $p<0,05$ compared to AS28.

DISCUSSION

This study evaluated the morphology and cardiac function in the cardiac remodeling process during the transition from dysfunction to heart failure in rats submitted to aortic stenosis and to physical training.

The main criterion for the diagnosis of ventricular dysfunction in experimental studies has been the level of final diastolic pressure of the LV, evaluated by the hemodynamic method [27]. However, its determination requires an invasive process, a fact that hinders longitudinal studies. Furthermore, the LV catheterization can cause aortic valve damage or affect cardiac performance [28]. Therefore, in our study, echocardiographic evaluation was utilized. The echocardiogram represents one alternative for the study of ventricular function and may offer important information on cardiac performance in rodents [27]; it allows evaluation of not only the morphological and functional evolution [28,29,30], but also the evolution of ventricular dysfunction caused by different types of aggression [31], and the effects of different interventions [32,33]. This method is versatile, safe, painless, noninvasive and relevant to analyses *in vivo* [34].

The present study showed that physical training for 10 weeks, initiated after 18 weeks of AS induction, provoked diminution of HF clinical signs, attenuated the structural cardiac remodeling and caused improvement of systolic and diastolic functions. These findings show that the physical training promoted atenuattion in cardiac remodeling that delayed the transition from ventricular dysfunction to heart failure.

The echocardiographic and anatomo-pathological data demonstrate that, after 18 weeks of AS there were important structural and functional cardiac alterations. Several parameters indicative of hypertrophy such as IVS and LVWT of LV, presented alterations that characterize concentric-type left ventricular hypertrophy. The functional analysis showed a drop in the posterior wall shortening velocity and a diastolic dysfunction evidenced by increased E/A and LA/Ao ratios. The structural and functional LV data, determined by echocardiogram and/or post-sacrifice, are in agreement with prior studies [9,10,25,35]. The data show ventricular dysfunction without the presence of HF signs, which indicates that these animals initiated training in a phase of transition from dysfunction to heart failure.

After the 10-week training period there was attenuation of structural alterations in the ASTR group in relation to AS28. There were diminutions in the right atrium and right ventricle/body weight ratios and the LV systolic and diastolic diameters. No studies were found in the literature that showed cardiac structural attenuation in rats with ventricular dysfunction with AS after physical training. However, Juric et al., 2007 [36], showed reversal of concentric hypertrophy and of diastolic dysfunction in rats with aortic stenosis after 2 weeks of treatment with resveratrol, a medicine with antioxidant properties.

The LV systolic function in AS28 group showed diminutions in endocardial fractional shortening and posterior wall shortening velocity. These data are in agreement with the observations of other authors after the 21st week of EAo induction [9,37,38]. The progressive loss of systolic function may be related to the following factors: 1) adverse geometric remodeling of the cavity [39,40] 2) structural alterations of the myocardium such as increase of extracellular matrix and/or diminution in the

number of myocytes, by necrosis or apoptosis [10,41,42]; 3) compromise of calcium transient and energetic balance that alters the contractile profile [42] or 4) a combination of these factors [10,41].

After the PT there was a rise in the endocardial fractional shortening and posterior wall shortening velocity, correlated with the diminution of LVSD. The restoration of systolic function may be related to improvement in one or more of the factors previously cited as being involved in the deterioration of cardiac function. Although our objective was not to evaluate the mechanisms responsible for improvement of ventricular function in the trained AS group, the geometric diminution of the LV cavity resulting in afterload reduction may be one of the mechanisms responsible for improvement of endocardial shortening. Our results are in agreement with the study of Jin et al., 2000 [43], who showed cardiac functional improvement in rats after acute myocardial infarction submitted to 13 weeks of endurance exercise. Wisloff et al., 2007 [44], showed in 27 patients with stable postinfarction HF, that 3 times per week, for 12 weeks of aerobic interval training, produced more beneficial than moderate continuous training the VO_2 peak and reverse LV remodeling. LV end-diastolic and end-systolic volumes declined only in aerobic interval training. Exercise intensity was an important factor for reversing LV remodeling and we agree that additional research is required to fully understand the real implication of exercise training intensity in the cardiac remodeling.

The diastolic dysfunction in group AS28 was evidenced by increases in the mitral E/A waves and LA/Ao ratios that may be related to alterations in elastic properties and disorders of intracellular calcium transient. Experimental studies have associated the augmentation of myocardial stiffness in AS with elevation of collagen fiber deposition

[10,45]. Alterations of proteins relative to reuptake of intracellular calcium, mainly the calcium pump of the sarcoplasmic reticulum, have also been related to a drop in diastolic performance in AS [10]. In the trained AS group there was a significant attenuation of this dysfunction. These alterations may have been partially reversed after training.

The modification in diastolic function associated with improvement in systolic function may be responsible for the amelioration of HF clinical signs observed after physical training in rats.

CONCLUSIONS

Physical training delayed the transition from ventricular dysfunction to heart failure in rats with aortic stenosis. There was attenuation of heart failure clinical signs and improvement of cardiac structure and function.

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ARTIGO 2

Effects of physical training on morphological and biochemical analysis, MRFs and IGF-I expression in rat skeletal muscle during the transition from cardiac hypertrophy to heart failure

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ABSTRACT

Objectives: The purpose of this study was to investigate the effects of physical training (PT) during the transition from cardiac hypertrophy to heart failure (HF) in soleus muscle of rats with aortic stenosis (AS), in relation to morphological and biochemical parameters and the expression of Myogenic Regulatory Factors (MRFs). **Methods:** There were 6 groups: aortic stenosis 18 weeks (AS18), Control 18 weeks (C18), aortic stenosis 28 weeks training (ASTR), aortic stenosis 28 weeks control (AS28), Control 28 weeks (C28), and Control 28 weeks training (TR). After 18 weeks of AS, when the animals show signal of ventricular dysfunction, they were submitted to PT for 10 weeks. The morphological aspects of fiber types and Myosin Heavy Chain (MHC) pattern, biochemical determination of TNF-α, Citrate Synthase activity (CS), IGF-I, MyoD, and myogenin gene expression and protein content were studied in soleus muscle. **Results:** HF promoted type IC/IIC fiber increase in AS28 compared to C28 and PT showed increased type I and decreased type IIa fibers in ASTR in relation to AS28. There were no significant differences in TNF-α levels, MyoD and myogenin gene and protein expression, CS activity, and IGF-I in HF and after PT. **Conclusions:** Physical training reversed soleus muscle phenotype alterations in rats with aortic stenosis, without altering MRF and biochemical analysis during transition from ventricular dysfunction to heart failure.

Key Words: aortic stenosis, heart failure, physical training, skeletal muscle.

INTRODUCTION

Heart failure (HF) is characterized by a reduced tolerance to exercise due to early fatigue and dyspnea; this in part may be due to skeletal muscle myopathy, with modifications in the proportion of myosin heavy chain (MHC) (Sullivan et al. 1990, Mancini et al. 1992, Vescovo et al. 1998, Simonini et al. 1999, De Sousa et al. 2000), oxidative metabolism (Lunde et al., 2001), and consecutive loss of muscle mass (Sullivan et al. 1990).

Recently in our laboratory, we found alterations in myogenic regulatory factor (MRF) expression in HF rats. The mRNA relative expression of MyoD in soleus and Extensor Digitorum Longus (EDL) muscles and of MRF4 in soleus muscle were significantly reduced, whereas myogenin did not change in either muscle from Wistar rats with monocrotaline-induced heart failure; thus demonstrating a potential role for MRFs in limb skeletal muscle myopathy during this syndrome (Carvalho et al. 2006).

Myogenic regulatory factors (MRFs) are a family of transcriptional factors that control the expression of several skeletal muscle specific genes (Hughes et al. 1993, Hughes et al. 1999). The family has four members: MyoD, myogenin, Myf5, and MRF4. MRFs form dimers with ubiquitous E proteins (e.g. E12 or E47) resulting in heterodimeric complexes that bind to the E-box consensus DNA sequence (5´-CANNTG-3´) found in the regulatory region of many muscle-specific genes (Murre et al. 1989). During embryogenesis, MRFs are critical for establishing myogenic lineage and controlling terminal differentiation of myoblasts (Parker et al. 2003). Several studies have suggested that MyoD transcript is prevalent in fast glycolytic muscle, whereas the myogenin transcript is mainly found in slow-oxidative muscle (Hughes et al. 1993). Studies have shown that myogenin is more involved with oxidative gene expression and

metabolic enzyme activity than contractile characteristics (Hughes et al. 1999, Ekmark et al. 2003, Siu et al. 2004).

The mechanisms that control MyoD expression during heart failure is still unknown; however, the influence of potential source neurohormones and cytokine activation has been reported. This last point has undergone considerable debate because tumor necrosis factor-alpha (TNF-α) is markedly increased in humans and animals with heart failure (Levine et al. 1990; McMurray et al. 1991, Anker et al. 1999). An imbalance between catabolic and anabolic systems has been observed in patients with HF and may be involved in skeletal muscle adaptations. Elevated TNF-α levels with decreased IGF-I serum levels have been described in patients with reduced tolerance to exercise and early fatigue in HF (Fan et al, 1995).

The effect of long-term physical training has been investigated in HF and shown to improve the functional capacity and quality of life (Coats et al 1992, Kiilavuori et al, 1996). Endurance training improves cardiovascular and muscle function. It is now accepted that an important component of the effects of physical training involves modifications to the skeletal muscle energy metabolism, and that these participate in the beneficial effects of training (Ventura-Clapier, 2009). Skeletal muscles adapt to repeated prolonged exercise by marked quantitative and qualitative changes in mitochondria and capillary supply, but only limited transitions in MHC isoforms (Fluck and Hoppeler 2003, Hood et al. 2006, Koulmann and Bigard 2006). It has been suggested that muscle improvement can be associated with a reversal of MHC content and fiber distribution. Results, however, are controversial. A shift towards type I fibers

was observed in only one study (Hambrecht et al, 1997), while other studies found no effect (Belardinelli et al, 1995; Kiilavuori, et al, 2000).

Physical training is indicated for patients with aortic stenosis (Bonow et al. 2005) however we found no studies evaluating the effects of the physical training factors controlling muscle phenotype characteristics in aortic stenosis induced HF in humans or in experimental model.

Based on these findings and previous studies we hypothesize that PT in rats with aortic stenosis 1) reverses fiber type distribution with alterations in MyoD, decreased TNF-α, and increased IGF-I; 2) regulates increases in metabolic oxidative enzyme, which parallel myogenin elevation.

METHODS

Experimental animals and study protocol

All experiments and procedures conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication no. 85-23, revised 1996; http://www.nap.edu/openbook. php?record_id=5140) and were approved by the Animal Ethics Committee (Sao Paulo State University, UNESP). Male Wistar weaning rats (3–4 weeks old), weighing 90–100 g, were anaesthetized with a mixture of ketamine (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Aortic constriction was created by placing a 0.6 mm i.d. stainless-steel clip on the ascending aorta via a thoracic incision, as previously described (Ding et al. 1999; Ribeiro et al. 2003). Control animals underwent left thoracotomy without clip placement (n = 24). The rats were housed in pathogen-free conditions at 23° C. They w ere exposed to a reverse light condition of 12 h of light and 12 h of darkness each day with food and water supplied *ad*

libitum. Eighteen weeks after surgery, part of Control animals (C18, n=4) and part of aortic stenosis animals (AS18, n=4) were sacrificed. Another part of Control and AS animals were divided in 4 groups: aortic stenosis 28 weeks training (ASTR, n=8), aortic stenosis 28 weeks control (AS28, n=6), Control 28 weeks (C28, n=9) and Control 28 weeks training (TR, n=6).

Physical training protocol

The training protocol utilized was modified from De Souza et al. 2002 and Siu PM et al. 2004. The animals in the ASTR and TR groups were submitted to a treadmill training program, five times per week, for ten weeks. The training protocol is described in Table 1.

Table 1. Physical training protocol

Weeks	Velocity (m/min)	Duration (min)
1	5	10
2	7,5	12
3	10	14
4	10	16
5	10	18
6	10	20
7	10	20
8	10	20
9	10	20
10	10	20

Anatomical Parameters

After anesthesia with intraperitoneal sodium pentobarbital (50 mg/kg), the rats were weighed and decapitated. Soleus muscles were dissected and immediately frozen in liquid nitrogen and stored at -80℃. Left ventri cle weight (LV), right ventricle weight (RV) and atrium weight (AT) were normalized by final body weight (LV/FBW, RV/FBW and AT/FBW respectively).

Clinical sign of HF

The presence of HF was evaluated, by tachypnea, and at rat sacrifice, by the presentation of pleural effusion, ascites, left atrium thrombi and hypertrophy of the right ventricle (Boluyt et al. 2005; Bregagnollo et al., 2006).

Histochemical and morphometrical analysis

Frozen Soleus (Sol) mid-belly regions were mounted vertically on a cryostat chuck in Tissue Freezing Medium (Jung, Germany). Transverse cryosections approximately 10 µm thick were cut in a cryostat cooled to -20°C. Sections were submitted to histochemical reaction for myofibrillar ATPase (m-ATPase) after acid pre-incubation at pH 4.32.

Electrophoretic separation of MHC

MHC isoform analysis was performed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Six to ten serial cross sections (12µm thick) were placed in 450µL of a solution containing 10% (wt/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, 2.3% (wt/vol) SDS, and 0.9% (wt/vol) Tris/HCl (pH6.8) for 10min at 60°C. Small amounts of the extracts (6µL) were loaded on a 7-10% SDS-PAGE separating gel with a 4% stacking gel, run overnight (19-21h) at 120V, and silver stained. MHC isoforms were identified according to molecular mass, and their relative percentages were quantified by densitometry.

RNA isolation, reverse transcription, and Real-Time PCR

Total RNA was extracted from Sol muscles with TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), which is based on the guanidine thiocyanate method. Frozen muscles were mechanically homogenized on ice in 1 mL ice-cold TRIzol reagent. Total RNA was solubilized in nuclease-free H_2O, incubated in DNase I (Invitrogen Life Technologies, Carlsbad, CA, USA) to remove any DNA present in the sample, and quantified by measuring the optical density (OD) at 260 nm. RNA purity was ensured by obtaining a 260/280 nm OD ratio of ~2.0.

For each sample, cDNA was synthesized from 2 µg of total RNA by using components from the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The reaction contained 10 µL 10X Reverse Transcription Buffer, 4 µL 25X dNTPs, 10 µL 10X random primers, 100 units of RNase inhibitor (Invitrogen Life Technologies, Carlsbad, CA, USA), 250 units of MultiScribe™ Reverse Transcriptase, and the final volume was adjusted to 100 µL with nuclease-free H_2O. The primers were allowed to anneal for 10 min at 25°C before the reaction proceeded for 2 h at 37°C. Control "No RT" reaction s were performed by omitting the RT enzyme. These reactions were then PCR amplified to ensure that DNA did not contaminate the RNA. The resulting cDNA samples were aliquoted and stored at -20°C. Two microliters of cDNA, corresponding to 20 ng of total RNA, from the RT reaction was used as a template in the subsequent real-time PCR, performed in a 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and the instrument's universal cycling conditions: 95°C for 10 min, 40 cycles of 9 5°C for 15 s and 60°C for 1 min. The reactions were run in duplicate using 0.4 µM of each primer and 2X Power SYBR Green PCR master mix (Applied Biosystems, Foster City, CA, USA) in a final volume of 25 µL.

Melting dissociation curves and agarose gel electrophoresis we re performed to confirm that only a single product was amplified. Control reactions were run lacking cDNA template to check for reagent contamination. Gene expression was compared in individual samples by using the Comparative CT Method described in Applied Biosystems User Bulletin No. 2. The TBP (Tata Box Binding Protein) was a housekeeping gene used to normalize the results.

Primers Design

Primer sequences were selected from the accession numbers in the National Center for Biotechnology Information database using the primer design function of the Primer Express v3.0 software (Applied Biosystems, Foster City, CA, USA) and were as follows:

MyoD (NM_176079.1) forward: 3'-TTTTTCATGCGACTCACAGC- 5', and reverse: 5'-GAAGGCAGGGCTTAAGTGTG- 3';

Myogenin (M24393) forward: 3'-GGAGTCCAGAGAGCGCCGTTGTTAA-5' and reverse: 5'- CGGTCGCGGCAGTCACTGTCTCT- 3';

IGF-I (NM_178866.2) forward 3'-GCTATGGCTCCAGCATTCG-5', and reverse 5'-TCCGGAAGCAACACTCATCC-3';

TBP (NM_001004198) and forward 3'- GCCACGAACAACTGCGTTGAT -5' and reverse 5'- AGCCCAGCTTCTGCACAACTCTA- 3'.

Western Blot

Muscles were lysed in assay lysis buffer containing freshly added protease and phosphatase inhibitors (1% Triton X-100, 100 mM Tris-HCl, pH 7.4, 100 mM sodium

pyrophosphate, 100 mM NaF, 10 mM sodium ortho-vanadium, 10 mM EDTA, 2 mM PMSF, and 10 mg/ml aprotinin). The samples were centrifuged for 20 min at 11,000 rpm, and the soluble fraction was resuspended in 50 ml Laemmli loading buffer (2% SDS, 20% glycerol, 0.04 mg/ml bromphenol blue, 0.12 M Tris-HCl, pH 6.8, and 0.28 Mm-mercaptoethanol). Then 50 mg of total protein homogenate from Sol was loaded on 8%–10% SDS-polyacrylamide gels. Proteins were transferred from the gels to a nitrocellulose membrane using a submersion electrotransfer apparatus (Bio-Rad Laboratories, Hercules, California). Membranes were blocked for 2 h at room temperature with 5% skim milk-Tris- HCl buffer saline-Tween buffer (TBS-T; 10 mM Tris- HCl, pH 8, 150 mM NaCl, and 0.05% Tween 20). The membranes were incubated with the primary antibodies overnight at 4℃, washe d in TBS-T, incubated with the peroxidase-conjugated secondary antibodies for 2 h at room temperature, and developed using the SuperSignal West Pico Chemiluminescent Substrate kit (Pierce Biotechnology, Rockford, Illinois). The beta actina was a housekeeping protein used to normalize the results. Band intensities were quantified using ImageJ 1.38X (National Institutes of Health, Bethesda, Maryland) software.

Antibodies Used for Western Blot

The following primary antibodies were used: (1) myogenin (rabbit polyclonal; Santa Cruz Biotechnology, Santa Cruz, California, USA); (2) MyoD (rabbit polyclonal; Santa Cruz Biotechnology, Santa Cruz, California, USA); (3) IGF- I (mouse monoclonal, Upstate Biotechnology, Lake Placid, New York, USA); (4) Beta Actina (mouse monoclonal; Santa Cruz Biotechnology, Santa Cruz, California, USA). The

corresponding secondary antibody used was horseradish peroxidase conjugated to mouse or rabbit IgG (HRP) (Santa Cruz Biotechnology, Santa Cruz, California, USA).

TNF-α analysis

At study entry, blood samples were taken, centrifuged at 3000 rpm for 15 min at 4ºC and supernatant separated and frozen at 80℃. S erum TNF-α level was measured by ELISA using a commercial kit (ELISA rat TNF-α ultra-sensitive- BioSource International, Camarillo, CA, USA). The procedures were performed according to the manufacturer's protocol.

Citrate Synthase activity

Citrate Synthase (CS, E.C.4.1.3.7.) activity (CS activity), an index of oxidative capacity (Spina et al. 1996) was determined for the Sol muscle of each rat (Bass et al. 1969). The Sol was removed and samples (200 mg) were weighed and homogenized in 5 mL of cold phosphate buffer (0.1 M, pH 7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA). A tissue homogenate was prepared in a motor-driven Teflon glass Potter Elvehjem tissue homogenizer (1 min 100 rpm) immersed in ice water. The homogenate was centrifuged at 10000 rpm for 15 min and supernatant was used for total protein and CS analysis (Bass, 1969). For CS activity the assay medium consisted of 50 mM Tris–HCl pH 8.1, 0.3 mM acetyl- CoA, 0.1 mM 5.5V-dithio-bis-2-nitrobenzoic acid (DTNB), and 0.5 mM oxaloacetate (omitted for control). Enzyme activities were determined using an ELISA reader (Bio-Tech Instruments, Inc., USA). The spectrophotometrics determinations were performed in a Pharmacia Biotech

spectrophotometer (974213, Cambridge, England). All reagents are from Sigma (Sigma, St. Louis, MO, USA).

Statistical analysis

The anatomical parameters were analyzed by the Student's t test for independent samples when the variable was shown to adhere to a normal probability distribution (data were expressed as mean ± standard deviation) and, by the non-parametric test of Mann-Whitney (data were expressed as median ± total semi amplitud) when this characteristic was absent.

Data from percentages of fibers type were compared using Goodman test (Goodman, 1964). MHC isoform percentages in the 18 and 28 weeks were compared using one-way analysis of variance followed by Bonferroni. MHC isoform percentages 28 weeks were compared using two-way analysis of variance followed by Tukey test (Zar, 1999).

MyoD expression are reported in median (maximum-minimum value), comparisons were made using Kruskal-Wallis analysis followed by Dunn analysis. Myogenin and IGF-I mRNA levels, myogenin, IGF-I and MyoD protein expression, TNF-α and CS analysis was presented as means ± S.D. Comparisons were made using measures analysis of variance followed by Bonferroni multiply comparisons.

Relationships between Myogenin and oxidative metabolism (CS activity) were examined by calculating the Pearson product-moment correlation coefficient, r. Significance was set at $p< 0.05$.

RESULTS

Clinical and anatomical parameters

Table 2 shows anatomical parameters from Control and AS groups, after 18 weeks of AS induction. There were left ventricular and atrial hypertrophy in the AS18 group in relation to C18. No anatomo-clinical signs of HF were observed in this period.

Table 2. Anatomical parameters from aortic stenosis 18 weeks (AS18, *n*=4) and control 18 weeks (C18, *n*=4) groups.

Parameters	Group	
	C18	**AS18**
Body Weight (g)	404 ± 61	470 ± 45
LV (g)	0,82 ± 0,19	1,35 ± 0,13*
LV/FBW (mg/g)	2,01 ± 0,17	2,88 ± 0,12*
RV (g)	0,25 ± 0,06	0,29 ± 0,02
RV/FBW (mg/g)	0,61 ± 0,09	0,61 ± 0,04
ATs (g)	0,11 ± 0,01	0,20 ± 0,05*
ATs/FBW (mg/g)	0,28 ± 0,01	0,42 ± 0,10*

FBW: final body weight; LV: total left ventricular weight; RV: right ventricular weight; ATs: atrium weight; Values are means ± SD. *p<0,05 compared to C18.

After 28 weeks of AS induction clinical signs of HF were observed such as tachypnea, ascites, pleural effusion, thrombus in the left atrium; whereas the ASTR group presented reduced intensity and frequency of these signs (Table 3). No alterations were found in control animals.

Table 3. Clinical data from aortic stenosis 28 weeks (AS28, *n*=6) and aortic stenosis 28 weeks with physical training (ASTR, *n*=8) groups.

HF signs	Groups	Relative frequency (%)
Tachypnea	AS28	100%
	ASTR	12,5%
Ascites	AS28	66%
	ASTR	25,5%
	ASTR	12,5%
Pleural Effusion	AS28	33%
	ASTR	33%
	AS28	50%
	AS28	16%
	ASTR	12,5%
Left atrium thrombi	ASTR	25%
	AS28	17%
	AS28	33%

In the AS28 group the anatomical parameters LV/FBW, RV/FBW and AT/FBW increased and after the exercise period, the animals of the ASTR group presented diminished in the RV/FBW and ATs/FBW ratios; there was no structural alteration in the LV (Table 4).

Table 4. Anatomical Parameters from control 28 weeks (C28, n=9), trained 28 weeks (TR, n=6), aortic stenosis 28 weeks (AS28, n=6) and aortic stenosis 28 weeks with physical training (ASTR, n=8) groups.

	Group			
Parameters	**C28**	**TR**	**AS28**	**ASTR**
Body Weight (g)	480 ± 44	436 ± 34	439 ± 59	426 ± 52
LV (g)	0,81 ± 0,07	0,84 ± 0,11	1,38 ± 0,29*	1,25 ± 0,19**
LV/FBW (mg/g)	1,70 ± 0,10	1,88 ± 0,17	3,10 ± 0,33*	2,92 ± 0,21**
RV (g)	0,27 ± 0,04	0,24 ± 0,05	0,52 ± 0,13*	0,30 ± 0,05$^{\#}$
RV/FBW (mg/g)	0,57 ± 0,07	0,54 ± 0,05	1,10 ± 0,27*	0,70 ± 0,11**$^{\#}$
ATs (g)	0,10 ± 0,01	0,10 ± 0,00	0,32 ± 0,13*	0,20 ± 0,08**$^{\#}$
AT/FBW (mg/g)	0,21 ± 0,02	0,22 ± 0,04	0,72 ± 0,25*	0,45 ± 0,15**$^{\#}$

FBW: final body weight; LV: left ventricular weight; RV: right ventricular weight; ATs: atrium weight; Values are means ± SD. * p<0,05 compared to C28; ** p<0,05 compared to TR; $^{\#}$ p<0,05 compared to AS28; $^{\#\#}$ p<0,05 compared to TR.

Histochemical and morphometrical analysis

Using the myofibrillar ATPase, after acid preincubations (4.32) three fibers types were identified in groups: dark staining fibers (type I), medium staining fibers (type IC/IIC) and pale staining fibers (type IIa) (Figure 1).

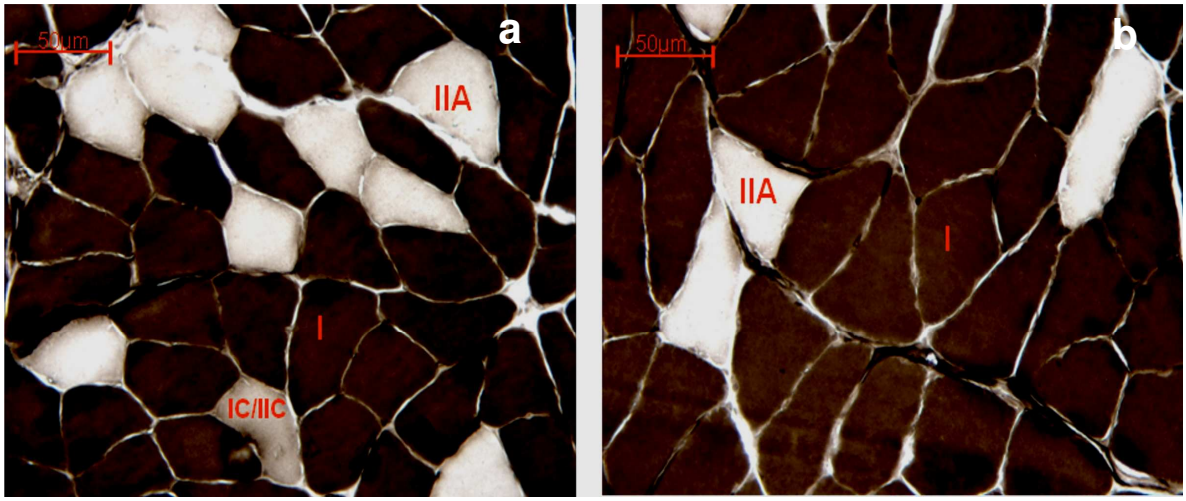

Fig 1. Transverse section of soleus muscle from AS28 (a) and CT28 (b) groups. Type I (I), type IC/IIC (IC/IIC) and type IIA (IIA) muscle fibers. Myofibrillar ATPase reaction, pH 4.32.

Muscle fibers type I decrease and type IIa increase and in C28 group compared to C18. There were a decreased of fiber type I and an increased of fibers type IC/IIC and IIa frequency in the AS28 group, compared with its corresponding AS18 group. Fibers type IC/IIC increased in AS28 compared to C28; (Table 5).

Ten weeks after the physical training, fibers type IC/IIC increased and type IIa decreased in TR group in relation to C28 group. Fibers type I increased and type IIa decreased in ASTR group in relation to AS28 group (Table 5).

Table 5. Fibers frequency distribution of soleus muscle from control 18 weeks (C18), aortic stenosis 18 weeks (AS18), control 28 weeks (C28, n=9), trained 28 weeks (TR, n=6), aortic stenosis 28 weeks (AS28, n=6) and aortic stenosis 28 weeks with physical training (ASTR, n=8) groups.

	Groups					
Fibers (%)	**C18**	**AS18**	**C28**	**AS28**	**TR**	**ASTR**
Type I	81,58	82,68	69,45$^{\#}$	63,24*	73,05	73,26**
Type IC/IIC	11,47	5,12	8,47	16,32*$^{\Delta}$	13,0$^{\#\#}$	13,86
Type IIa	6,95	12,20	22,08$^{\#}$	20,44*	13,95$^{\#\#}$	12,88**

Values are means in relation to total type fibers. *p< 0,05 compared to AS18.$^{\Delta}$compared to C28; $^{\#}$compared to C18; **compared to AS28; $^{\#\#}$ compared to C28;

MHCs electrophoretic pattern

In Sol muscle, two MHC isoforms were separated, MHC I and MHC IIa. MHC I decreased and MHC IIa increased in the C28 group compared to C18; this result was similar in the group AS28 compared to AS18.

In relation to physical training, MHC I increased and MHC IIa decreased in the TR group compared to C28. MHC I were lower and MHC IIa were larger in ASTR than TR. MHC I and MHC IIa were similar in AS and ASTR groups (Table 6).

Table 6. Myosin Heavy Chain (MHC) frequency of soleus muscle from control 18 weeks (C18), aortic stenosis 18 weeks (AS18), control 28 weeks (C28, n=9), trained 28 weeks (TR, n=6), aortic stenosis 28 weeks (AS28, n=6) and aortic stenosis 28 weeks with physical training (ASTR, n=8) groups.

	Groups					
MHC %	**C18**	**AS18**	**C28**	**AS28**	**TR**	**ASTR**
MHC I	77,02±8,05	78,04±4,93	67,26±3,87*	67,38±9,16**	75,21 ± 7,81***	67,18 ± 6,96#
MHC II	22,98±8,05	21,96±4,93	32,74±3,8*	32,62±9,16**	24,79 ± 7,81***	32,82 ± 6,96#

Values are means ± SD. * p<0,05 compared to C18; ** compared to AS18; *** compared to C28; # compared to TR.

MyoD, Myogenin e IGF-I mRNA levels estimated by Real-Time PCR

MyoD mRNA levels were larger in the AS28 than AS18 group. Myogenin mRNA levels were lower in the AS28 than AS18 group. There were no significant differences in the others groups.

IGF-I expression was similar in the groups (Table 7).

Protein Levels of MyoD, Myogenin and IGF-I

MyoD and Myogenin protein expression was similar in the groups. IGF-I protein concentration were lower in the C28 than C18 group. The IGF-I protein concentration were lower in the AS28 than AS18 group; (Table 7).

Table 7. MyoD, myogenin and IGF-I gene expression and protein content of soleus muscle from control 18 weeks (C18), aortic stenosis 18 weeks (AS18), control 28 weeks (C28, n=9), trained 28 weeks (TR, n=6), aortic stenosis 28 weeks (AS28, n=6) and aortic stenosis 28 weeks with physical training (ASTR, n=8).

	Groups					
Parameters	**C18**	**AS18**	**C28**	**AS28**	**TR**	**ASTR**
MyoD-G	0,44(0,2-0,6)	0,25(0,2-0,4)	0,47(0,2-8,5)	0,98(0,4-1,1)*	0,47(0,2-0,9)	0,69(0,3-1,5)
MyoD-P	1,05 ± 0,27	1,16 ± 0,07	1,38 ± 0,61	0,79 ± 0,28	0,93 ± 0,38	0,92 ± 0,29
Myogenin-G	1,10 ± 0,24	1,58 ± 0,42	0,75 ± 0,31	0,84 ± 0,53*	1,04 ± 0,47	0,7 ± 0,23$^{\#}$
Myogenin-P	0,94 ± 0,28	1,20 ± 0,46	1,46 ± 0,70	0,75 ± 0,28	1,04 ± 0,34	0,97 ± 0,28
IGF-I-G	0,72 ± 0,54	0,71 ± 0,12	0,65 ± 0,18	0,70 ± 0,31	0,86 ± 0,43	0,67± 0,24
IGF-I-P	1,54 ± 0,12	1,63 ± 0,05	0,94 ± 0,3**	0,76 ± 0,15$^{\#\#}$	1,04 ± 0,13	0,79 ± 0,25

G: gene expression; P: protein expression; Values are means ± SD. MyoD= median (maximum - minimum value); *p<0,05 compared to AS18, $^{\#}$ compared to AS18; **compared with C18, $^{\#\#}$ compared with AS18.

TNF-α level

There were no significant differences in TNF-α level in the groups (Table 8).

Citrate Synthase Activity

There were no significant differences in Citrate Synthase activity in the groups (Table 8).

Table 8. Biochemical data from control 18 weeks (C18), aortic stenosis 18 weeks (AS18), control 28 weeks (C28, n=9), trained 28 weeks (TR, n=6), aortic stenosis 28 weeks (AS28, n=6) and aortic stenosis 28 weeks with physical training (ASTR, n=8).

Parameters	Groups					
	C18	**AS18**	**C28**	**AS28**	**TR**	**ASTR**
TNF-α (pg/ml)	67 ± 0,15	84 ± 0,07	86 ± 0,22	69 ± 0,16	65 ± 0,32	70 ± 0,23
CS (U/100mg p)	3,34 ± 1,47	2,37 ± 0,61	3,98 ± 1,90	3,62 ± 0,72	3,94 ± 1,38	3,53 ± 0,71

TNF-α: tumor necrosis factor; CS: Citrate Synthase activity; p: protein.

Relashionships between Myogenin and Citrate Syntase Activity

We found that the myogenin gene expression and protein content was negatively correlated with the CS activity (Figures 2 and 3).

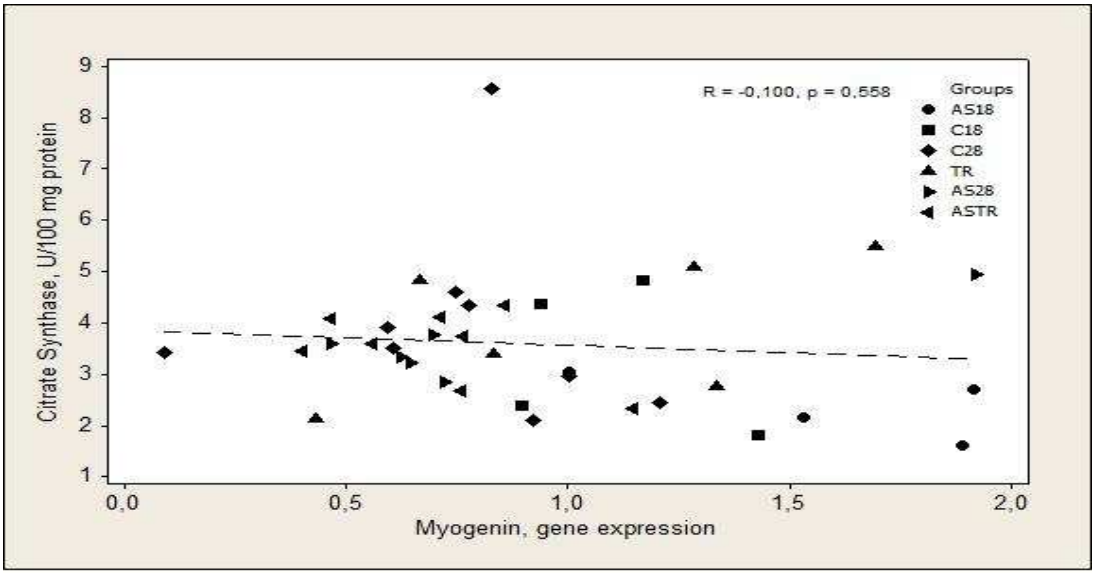

Fig 2. Relationship between the myogenin gene expression and CS activity. Control 18 weeks (C18), aortic stenosis 18 weeks (AS18), control 28 weeks (C28, n=9), trained 28 weeks (TR, n=6), aortic stenosis 28 weeks (AS28, n=6) and aortic stenosis 28 weeks with physical training (ASTR, n=8).

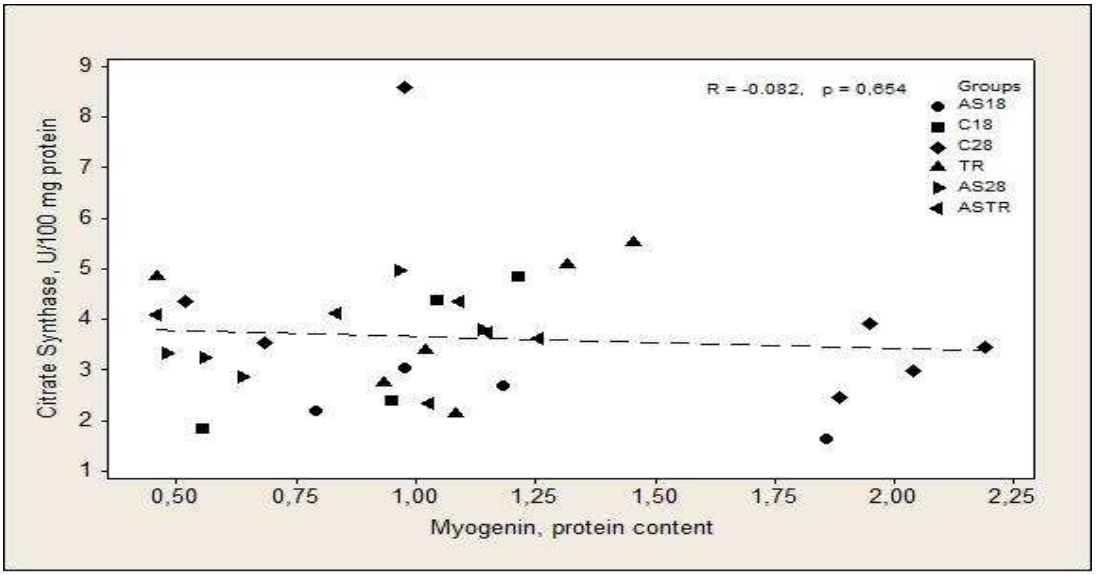

Fig 3. Relationship between the myogenin protein content and CS activity. Control 18 weeks (C18), aortic stenosis 18 weeks (AS18), control 28 weeks (C28, n=9), trained 28 weeks (TR, n=6), aortic stenosis 28 weeks (AS28, n=6) and aortic stenosis 28 weeks with physical training (ASTR, n=8).

DISCUSSION

The purpose of this study was to investigate the effects of PT during the transition between cardiac hypertrophy and HF in soleus muscle of rats with aortic stenosis in relation to morphological and biochemical parameters and MRF expression. The major finding in this study is that HF induces the transition from slow to fast fibers without altering MRFs and that 10 weeks PT led to a decrease in type IIa and a rise in type I muscle fibers reverting soleus phenotype without altering MyoD, myogenin and the possible mechanisms involved in these MRFs.

In our study, HF promoted a transition from slow to fast fibers. Temporary alterations also were observed in C18 and AS18 compared to C28 and AS28 groups; this showed an increase in type IIa fiber and a decrease in type I fiber frequencies.

Hybrid fibers (IC/IIC) only increased in AS28 compared to AS18. These alterations were accompanied by changes in MHC composition (decreased MHC I and increased MHC IIa). A study in our laboratory has shown increased MHC IIa and type IIa fibers and decreased MHC I and type I fibers in soleus muscle during late cardiac hypertrophy (18 weeks) and twenty-eight weeks after AS (Carvalho et al. 2003). The difference between results may be related to heart failure severity (Toth et al. 2004). In the AS experimental model, animals can develop HF with different degrees of ventricular dysfunction which can be evaluated by echocardiogram. Moreira et al. 2006 observed that, 28 weeks after AS, rats with mild or severe cardiac dysfunction may be characterized by anatomical and functional cardiac parameters. So it is possible that in the studies of Carvalho et al., 2003 cardiac function was more altered than in our study (Lopes et al. 2009). On the other hand, temporary alterations may be related to fiber modulations that occur during muscle growth (Navarrete and Vrbová, 1983).

The most important finding in this study was that physical training led to a decrease in type IIa and an increase in type I muscle fibers in ASTR compared to AS28, without altering MyoD, myogenin, and the possible mechanisms involved in these MRFs. These results demonstrated that the proposed PT promoted muscle phenotype modulation (fast to slow), without altering MHC composition and oxidative metabolism, the last parameter analyzed by CS synthase activity. The changes in contractile characteristics found in our experiment are similar to those seen by De Souza et al. 2002. They showed that 8 weeks of voluntary exercise changed the contractile capacity of soleus muscle in rats 4 months after AS induction. They evaluated MHC and metabolic aspects in the soleus muscle. Sedentary AS animals presented a rise in MHC IIa and a drop in MHC I, and a decrease in oxidative metabolism. The AS group

submitted to voluntary low-intensity exercise presented normalization of MHC contents and some metabolic parameters; however CS activity did not show changes similar to those in our experiment. Similar results were seen by Hambrecht et al., 1997 in the gastrocnemius muscle of humans with HF after six months endurance training. However, several authors have shown that in patients with stable HF, a physical training program did not change MHC distribution (Belardinelli et al. 1995, Kiilavuori et al. 2000, Harjola et al. 2006). Although there was been a degree of interpretation bias in results concerning the effect of PT on skeletal muscle in HF, we think that PT in the presence of left ventricular dysfunction can retard muscular alterations in AS promoted HF; and may be considered a non-pharmacological measure to improve the functional capacity and quality of life in this patients.

Comparing TR and C28 groups, myofibrillar ATPase analysis showed an increase in hybrid fibers (IC/IIC) and a decrease in IIa fibers in TR, a fact confirmed by electrophoresis analysis which showed an increase in MHC I and decrease in MHC II. This confirmed that PT was efficient at modulating soleus muscle phenotype toward slow type, even in the absence of AS.

Several studies have shown that correlation may exist between histochemical muscle fiber differentiation (ATPase) and electrophoresis identification of MHC in skeletal muscle (Bee et al. 1999). However our ATPase analysis did not correlated with MHC analysis in all groups. This may be related to the separation of Soleus myosin isoforms into two bands, I and II, given that the hybrid isoforms (IC/IIC) can migrate to any of the bands. Most works involving Soleus muscle electrophoresis were able to separate MHCs into two bands: I and IIa, thus thwarting the separation of hybrid isoforms (Vescovo et al 1998, Carvalho et al. 2003).

In relation to MRFs, no alterations were observed in gene and protein expression in our experiment. There was a decrease in myogenin and increase in MyoD gene expression only in AS28 compared to AS18.

MyoD and myogenin are MRFs that act as key regulatory molecules during early muscle differentiation; they may play a more extensive role because they are also expressed in postmitotic mature muscles (Hughes et al. 1993). Several studies suggest that MRFs are involved in regulating the metabolic processes intrinsic to muscle catabolism or anabolism (Hughes et al. 1993, Dupont et al. 1998, Mozdziak et al. 1998). Myogenin is expressed at higher levels than MyoD, predominantly in slow muscle, whereas MyoD is expressed at higher levels than myogenin, mostly in fast muscles of adult animals (Hughes et al, 1993). Additionally, myogenin is also involved in oxidative gene expression and metabolic enzyme activity (Hughes et al. 1999, Ekmark et al. 2003, Siu et al. 2004).

Studies by our group have demonstrated alterations in MRF gene expression in rats with monocrotaline induced HF (Carvalho et al. 2006, Lopes et al 2008). Despite relative MyoD mRNA expression being significantly reduced in soleus and extensor digitorum longus (EDL) muscles while myogenin did not change, no changes in MHC composition were observed (Carvalho et al. 2006). Another study showed that HF decreased the relative MyoD mRNA level without changes in mRNA relative myogenin expression in diaphragm muscle. However the authors did not evaluate myogenin and MyoD protein expression. The reason for these discrepancies is unclear but may be related to differences in muscle type or HF model used.

Although the mechanisms that control MyoD expression in skeletal muscle in HF were not determined, studies have demonstrated that inflammatory cytokines such as

TNF-α influence its expression (Israël 2000). One hallmark of TNF-α action is activation of nuclear factor Kappa B (NFκB), a ubiquitous transcription factor that can down-regulate MyoD mRNA at a post-transcriptional level (Baldwin 1996, Israël 2000). There is also an increase in TNF-α serum concentrations, which may partially explain the down-regulation of MyoD (Carvalho et al. 2010). Our MyoD results were consistent with this TNF-α analysis, both inaltered. Elevated TNF-α levels with decreased IGF-I serum levels have been described in patients with HF (Fan et al. 1995, Hambrecht et al. 2005). However, an imbalance between catabolic (TNF-α) and anabolic (IGF-I) systems was not observed in this study. In our experiment IGF-I protein expression decreased, type I muscle fiber frequency and MHC I content decreased without altering TNF-α in AS28 compared to AS18. This alteration over time may be related to aging adaptation (Giovannini et al. 2008). Tanner et al. 2007 also did not observe elevated TNF-α levels in patients with stable and moderate HF. Disease severity is related to the magnitude of cytokine activation in advanced heart failure (Maeda et al. 2000).

Modifications in MRFs and TNF-α in other studies may be related to the model used to induce HF. The peripheral and cardiac alterations found in this model result from faster HF installation (22 days with HF) and elevated pulmonary hypertension, a characteristic sign in this model (Vescovo et al. 1998, Dalla Libera et al. 2001). In our experiment AS model alterations were more gradual and less aggressive, as revealed in morphological analysis and echocardiogram (18 weeks with cardiac dysfunction and 28 weeks with HF) (Moreira et al. 2006). Based on the results shown here, we agree that additional research is required to fully understand the real implications of MRF fiber regulation and MHC transitions during HF.

The IGF-I gene expression in our experiment did not change, but IGF-I protein expression did decrease in AS28 compared to AS18. Given that the protein expression change in our study may have been due to a number of posttranscriptional controls (e.g., RNA splicing, RNA editing, blocked nuclear export, subcellular location, negative translational control), a close relationship between mRNA and protein would not be expected (Moore, 2005).

The proscribed PT did not alter MRFs or muscular oxidative profile. We did not find any studies that evaluated the effects of PT on MRFs with HF. Myogenin did not alter in parallel with citrate synthase activity. In contrast to our study, Siu et al., 2004, observed a rise in myogenin associated with increased oxidative metabolism after 8 weeks treadmill endurance training at 28m/min in soleus muscle from healthy animals. The effort in PT intensity was exceptionally high compared to that used in our experiment, possibly the crucial factor in their finding positive correlation between myogenin and oxidative metabolism. Yang et al. 2004 showed time course of myogenic gene expression in response to acute exercise in human skeletal muscle; the authors showed an increase in both myogenin and MyoD mRNA transcripts for resistance and only MyoD mRNA transcripts in running exercises. Gene induction timing was variable, with peak gene expression occurring 4–8h after exercise session. In this study all mRNA levels were not significantly different from pre-exercise levels within the 24h after the exercise session, and no induction was observed for myogenin gene over the 24h after running exercise sessions. Harber et al. 2009 examined the acute response of two distinctly different leg muscles (vastus lateralis and soleus) from eight men before and after a 45 min level treadmill run. At the transcriptional level, the soleus muscle appears slightly more responsive to acute running exercise than the vastus lateralis. MyoD gene

expression increased 4h after the exercise and no change after 24h. Overall, these data indicate a muscle specific gene expression response in hours immediately after running exercise, which suggest that muscle specific differences in adaptation are possible in response to training.

These data could help to explain our results (chronic training) and provide a basic timeline influence for MRF regulation with PT and training model used. Thus, the time course for MRFs and fiber type-specific alterations that occur during PT in HF need to be further clarified.

CONCLUSION

PT in rats with aortic stenosis was shown to be a beneficial non-pharmacological measure to reverse soleus muscle phenotype alterations in rats with aortic stenosis, without changes in MRFs and biochemical analysis during the transition from ventricular dysfunction to heart failure.

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6. CONCLUSÕES GERAIS

Após 18 semanas de estenose aórtica supravalvar em ratos ocorreu remodelação cardíaca com alterações anatômicas e funcionais, porém, sem alterações no músculo esquelético soleus. Houve hipertrofia concêntrica constatada por aumento da espessura do septo interventricular, da parede posterior do ventrículo esquerdo e da relação ventrículo esquerdo/peso corporal. A análise funcional monstrou diminuição da velocidade de encurtamento da parede posterior do ventrículo esquerdo e disfunção diastólica caracterizada por aumento das relações ondaE/ondaA e átrio/peso corporal. Estas alterações indicam que estes animais estavam em um momento de transição entre disfunção ventricular e insuficiência cardíaca.

Com a progressão do quadro patológico constatou-se a insuficiência cardíaca com 28 semanas após indução da estenose aórtica. A IC foi avaliada pela observação in vivo da presença da dispnéia e pelo aparecimento de retenção hídrica e alterações anatômicas avaliadas pós morte: ascite, derrame pleural e trombo em átrio esquedo e hipertrofia de ventrículo direito (relação ventrículo direito/peso corporal). Houve alterações do fenótipo muscular (para rápido), sem alterar o metabolismo oxidativo, os fatores de regulação miogênicos MyoD e Miogenina, IGF-I e TNF-α. Houve agravamento funcional e anatômico da remodelação cardíaca. A função sistólica piorou como mostrada pela diminuição da velocidade de encurtamento da parede posterior e fração de encurtamento do endocárdio. Houve prejuízo também ao relaxamento ventricular (função diastólica) com aumento das relações ondaE/ondaA e átrio/peso corporal.

Na remodelação cardíaca o treinamento físico promoveu diminuição de parâmetros anatômicos cardíacos (relações átrio/peso corporal e ventrículo direito/peso corporal e dos diâmetros sistólico e diastólico do ventrículo esquerdo), melhora nas funções sistólica (aumento da fração de encurtamento do endocárdio e velocidade de encurtamento da parede posterior) e diastólica (diminuição das relações ondaE/ondaA e da relação átrio esquerdo/aorta) do ventrículo esquerdo. Houve também melhora clínica com diminuição dos sinais de IC (taquipnéia, ascite, derrame pleural e trombo em átrio esquerdo). Os mecanismos envolvidos nesta melhora não foram avaliados, mas alterações na matriz extracelular com modificações do colágeno, melhora do trânsito de cálcio, diminuição de perdas de cardiomiócitos podem estar envolvidas.

No músculo esquelético soleus a IC modificou o padrão contrátil muscular de lento para rápido com aumento das fibras híbridas do tipo IC/IIC no grupo estenose aórtica 28 semanas comparado com o grupo estenose aórtica 18 semanas. Mas, não houve alterações dos fatores de regulação miogênicos: MyoD e miogenina e seus mecanismos envolvidos (TNF-α, IGF-I e metabolisno oxidativo).

O treinamento físico promoveu alteração no padrão contrátil do músculo sóleo com diminuição das fibras IIa e aumento das fibras I no grupo estenose aórtica treinado comparado com o grupo estenose aórtica 28 semanas. Isto demonstra que o treinamento físico alterou o fenótipo muscular de r[apido para lento, porém, sem alterar o metabolismo oxidativo, e os fatores de regulação miogênicos MyoD e miogenina.

Os MRFs contribuíram para a miopatia (nos músculos diafragma, soleus e extensor longo dos dedos) na IC induzida por monocrotalina, em experimentos prévios do nosso laboratório. Este modelo de IC caracteriza-se por aumento da resistência vascular pulmonar, com hipertrofia de ventrículo direito e insuficiência cardíaca direita,

de instalação rápida (22 dias). Provavelmente as características da IC, como gravidade da doença, são distintas nestes modelos de indução da IC, o que pode ter ocasionado essas diferentes respostas.

Outro fator que pode ter contribuído para a não alteração dos MRFs foi o momento em que foi feita a análise da expressão gênica e protéica. Este estudo buscou avaliar os efeitos crônicos do treinamento físico, e a eutanásia dos animais foi realizada após os animais completarem 10 semanas de treino e esperou-se 72 horas após a última sessão de exercício. Muitos estudos evidenciam a participação destes fatores de regulação miogênicos 4-8 horas após a finalização do exercício, com estabilização de expressão gênica e protéica em até 24 horas. Isto demonstra uma possível resposta temporal destes fatores, indicando uma participação mais aguda pós exercício.

Um fator que pode ter contribuído para não ocorrência do aumento do metabolismo oxidativo associado ao aumento da miogenina foi a intensidade do treinamento físico proposto.

Este estudo indica que embora alterando o fenótipo muscular com o treinamento físico na IC, os MRFs parecem não estar envolvidos nesta transição fenotípica. Sugere-se mais estudos que possam investigar quais os mecanismos de controle fenotípico na IC e após a aplicação de treinamento físico.

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DECLARAÇÃO

Declaro para os devidos fins que o conteúdo de minha Tese de Doutorado intitulada "Efeitos do treinamento físico na remodelação cardíaca e músculo esquelético durante a transição entre disfunção ventricular e insuficiência cardíaca":

( ) não se enquadra no § 3º do Artigo 1º da Informação CCPG 01/08, referente a bioética e biossegurança.

(X) tem autorização da(s) seguinte(s) Comissão(ões) de Bioética ou Biossegurança*:
Comissão de Ética na Experimentação Animal (CEEA), sob Protocolo(s) nº 57/07-CEEA.

** Caso a Comissão seja externa à UNICAMP, anexar o comprovante de autorização dada ao trabalho. Se a autorização não tiver sido dada diretamente ao trabalho de tese ou dissertação, deverá ser anexado também um comprovante do vínculo do trabalho do aluno com o que constar no documento de autorização apresentado.*

Aluna: Francis Lopes Pacagnelli

Orientadora: Maeli Dal Pai Silva

Para uso da Comissão ou Comitê pertinente:
(X) Deferido ( ) Indeferido

Nome: Profa. Dra. ANA MARIA A. GUARALDO
Função: Presidente
Comissão de Ètica na Experimentação Animal
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Campus de Botucatu

CERTIFICADO

Certificamos que o Protocolo nº **57/07-CEEA**, sobre "Expressão dos fatores de regulação miogênica no músculo estriado esquelético de ratos com insuficiência cardíaca submetidos ao exercício: relação metabólica, hormonal e inflamatória", sob a responsabilidade de **MAELI DAL PAI SILVA**, está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Colégio Brasileiro de Experimentação Animal (COBEA) e foi aprovado pela ***COMISSÃO DE ÉTICA NA EXPERIMENTAÇÃO ANIMAL*** (CEEA), em reunião de 17/08/2007.

Botucatu, 17 de agosto de 2007.

Prof. Dr. MARCELO RAZERA BARUFFI
Presidente - CEEA

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