ANDREY JORGE SERRA

EFEITO CARDIOPROTETOR DO EXERCÍCIO FÍSICO NA SOBRECARGA β-ADRENÉRGICA CARDÍACA INDUZIDA POR ISOPROTERENOL

Tese Apresentada à Disciplina de Cardiologia da Universidade Federal de São Paulo / Escola Paulista de Medicina para Obtenção do Título de Doutor em Ciências

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Orientador: Prof. Dr. Paulo José Ferreira Tucci

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INTRODUÇÃO

O coração é um órgão capaz de remodelar-se em resposta à grande variedade de estímulos. Estímulos como o exercício físico e a gravidez induzem remodelamento cardíaco fisiológico, em que estrutura e função cardíaca permanecem preservadas. Todavia, estímulos como o infarto do miocárdio e a hipertensão arterial promovem remodelamento cardíaco patológico, em que anormalidades metabólicas, gênicas, estruturais e funcionais são frequentemente presentes (Hill & Olson, 2008).

O remodelamento patológico do coração tem sido apontado como poderoso marcador de risco para desenvolvimento de insuficiência cardíaca e arritmias (Kannel et al, 1987a; Lvy et al 1990; Lips et al, 2003; Hill & Olson, 2008). Tais constatações conferem à hipertrofia miocárdica (HM) papel de grande relevância para aumento do risco de morbi-mortalidade cardiovascular (Kannel et al, 1969; Kannel et al, 1987a; Kannel et al, 1987b; Levy et al, 1990), talvez, mais importante que outros fatores de risco tradicionais como tabagismo, dislipidemia e hipertensão arterial (Braunwald, 1991).

Há fortes evidências que apóiam a existência de estrita relação entre HM e atividade adrenérgica cardíaca exacerbada (Zierhut & Zimmer, 1989; Fleming et al, 1992; Zou et al, 1999; Brum et al, 2002). Esta sabida interação entre atividade adrenérgica e HM já ficou bem caracterizada em ensaios clínicos. Em sujeitos hipertensos acometidos por HM, os níveis de noradrenalina circulante e a frequência de descarga simpática em nervos periféricos estavam elevados, quando comparados com sujeitos hipertensos, mas sem HM e, com níveis de pressão arterial similares (Greenwood et al, 2001; Schlaich et al, 2003). Além disso, em estudo prospectivo conduzido em pacientes hipertensos acompanhados por 20 anos, Strand et al (2006) mostraram que os

níveis plasmáticos basais de noradrenalina são fortes preditores do grau de HM e, isto pareceu ocorrer independentemente dos níveis de pressão arterial sistólica.

Em que pese a importância da atividade adrenérgica para o desenvolvimento de HM, ativação pronunciada do sistema nervoso simpático na insuficiência cardíaca tem sido pontuada como determinante de piora da progressão da doença, aumento de morte súbita e sobrevida reduzida (Cohn et al, 1984; Packer 1988; Francis et al, 1993; Brunner-la Rocca et al, 2001; Dorn, 2002; Floras 2003, Grassi et al, 2009). Kaye et al (1995) observaram que pacientes com insuficiência cardíaca apresentaram piora no prognóstico da doença associada com aumento da atividade do sistema nervoso simpático. No mesmo estudo, a taxa de liberação cardíaca de noradrenalina foi identificada como poderoso marcador de mau prognóstico. Estes dados da literatura conduziram à racionalidade clínica para o uso de beta-bloqueadores como parte do tratamento padrão da insuficiência cardíaca (Chatteriee, 2002; Hunt et al, 2005).

Sob a luz dos estudos clínicos, modelos experimentais de administração crônica de agonistas beta-adrenérgicos constituem abordagem interessante para compreensão da fisiopatologia cardíaca inerente à ativação adrenérgica sustentada. Para este fim, o modelo experimental de sobrecarga adrenérgica com isoproterenol (ISO) é comumente encontrado na literatura. Primariamente, o ISO foi usado em altas doses com o intuito de padronizar um modelo experimental equivalente ao infarto do miocárdio (Rona et al, 1959; Handforth, 1962; Rona, 1963; Milei et al, 1978; Milei et al, 1979). Naquela época, considerando os mecanismos envolvidos na indução da injúria e de HM secundárias à administração do ISO, intuía-se que a vasodilatação sistêmica, com hipotensão e consequente hipóxia tecidual, somados com o aumento do consumo de oxigênio pelos

cardiomiócitos e sobrecarga intracelular de cálcio, eram os fatores causadores de anormalidades. Em outro momento, o modelo de ISO foi postulado como paradigma para estudos de HM. Nesta perspectiva, Stanton & Bowman (1967) avaliaram a relação dose/efeito e verificaram que o montante de HM é proporcional à dose utilizada de ISO. Os autores observaram que doses de ISO superiores a 5 mg/kg de peso corporal não resultaram em aumentos adicionais da massa miocárdica. Posteriormente, Taylor & Tang (1984a) descreveram aumento máximo da massa miocárdica com oito dias de administração de 0,3 mg/kg de peso corporal de ISO. Em recente revisão da literatura, Osadchii (2007a) documentou aumentos na ordem de 30% a 70% na massa miocárdica de animais submetidos a tratamento prolongado com doses de ISO, que variaram entre 0,02 a 3,0 mg/kg de peso corporal.

O emprego de técnicas de biologia molecular e de métodos histoquímicos permitiu outros esclarecimentos acerca das modificações cardíacas promovidas pelo ISO, além de aumento da massa miocárdica. Assim, estruturalmente, a administração de ISO resultou em espessamento das paredes do ventrículo esquerdo com aumento do diâmetro celular (Alderman & Harrison, 1971; Zou et al, 1999; Leenen, 2001; Gao et al, 2009). Estas modificações foram acompanhadas por acentuada perda de cardiomiócitos e exacerbado acúmulo de tecido fibroso (Rona et al, 1959; Bloom & Cancilla, 1969; Bloom & Davis, 1972; Milei et al, 1978; Taylor & Tang, 1984b; Benjamin et al, 1989; Teerlink et al; 1994; Shizukuda et al, 1998; Grimm et al, 1998; Dudnakova et al, 2002; Ennis et al, 2003; Zhang et al, 2005; Prabhu et al, 2006).

Atualmente, múltiplas vias de sinalização parecem estar envolvidas com a lesão tecidual e HM derivadas do uso de ISO, incluindo: superexpressão de proto-oncogenes

(Brand et al, 1993; Saadane et al, 2000), formação de espécies reativas de oxigênio (Rathore et al, 1998; Zhang et al., 2005; Ishizawa et al, 2006; Zhou et al, 2006), ativação do sistema renina-angiotensina-aldosterona (Nagano et al, 1992; Gallego et al, 2001; Oliveira & Krieger, 2005), sobrecarga de cálcio intracelular (Bloom & Davis, 1972; Mann, 1998), aumento na liberação local de citocinas pro-inflamatórias (Murray et al, 2000; Chandrasekar et al, 2004), ativação de proteínas quinases ativadoras de mitogenia (Sugden & Bogoyevitch, 1995; Zou et al, 2001), ativação da via da fosfatídilinositol 3-quinase (Morgan & Baker, 1991; Chandrasekar et al, 2004) e fator transformador de crescimento β_1 (TGF-β_1) (Boluyt et al, 1995). Genes normalmente expressos somente no período fetal, como fator natriurético atrial (Boluyt et al, 1995; Oliveira & Krieger, 2005; Saadane et al, 2000), alfa actina esquelética (Saadane et al, 2000), fibronectina (boluyt et al, 1995) e cadeia pesada beta da miosina (Boluyt et al, 1995; Saadane et al, 2000), também foram mostrados estar superexpressos no miocárdio de roedores tratados com ISO.

Em relação à função cardíaca, uma série de estudos não relatou prejuízos apreciáveis decorrentes do tratamento com ISO (Taylor et al, 1989; Baldwin et al, 1982; Osadchii et al, 2005) e, alguns investigadores, caracterizaram função cardíaca hiperdinamica (Taylor & White, 1983; Taylor & Tang, 1984a; Tang et al, 1987; Tang & Taylor 1996; Oliveira & Krieger, 2005).

Relatos análogos que avaliaram a capacidade de bombeamento cardíaco evidenciaram função ejetante deprimida associada ao ISO (Anderson et al, 2008; Kazachenko et al, 2008; Schumacher et al, 2008). De fato, indicações consistentes apontaram que o ISO pode induzir disfunção sistólica e/ou diastólica do ventrículo

esquerdo (Beznak & Hacker, 1964; Jalil et al, 1989; Teerlink et al, 1994; Grimm et al, 1998; Grimm et al, 1999; Murad & Tucci, 2000; Woodiwiss et al, 2001; Oudit et al, 2003; Osadchii et al, 2007b; Krenek, 2009). Somam-se a isto, opiniões equivalentes de depressão da capacidade contrátil, avaliada em estudos de preparações musculares isoladas (Hayes et al, 1986; Vassallo et al, 1988; Stein et al, 1996).

A acentuada perda de cardiomiócitos com conseqüente aumento do conteúdo de colágeno (Jalil et al, 1989), alterações na expressão gênica (Boluyt et al, 1995; Linck et al, 1998) e protéica (Stein et al, 1996; Linck et al, 1998; Menge et al, 2006) de biomoléculas envolvidas com o ciclo de contração/relaxamento muscular e modificações no estado de fosforilação de proteínas envolvidas com o ciclo do cálcio (Stein et al, 1996 Meng et al, 2006) tem sido postulados como possíveis fatores envolvidos com as alterações das propriedades funcionais cardíacas promovidas pelo ISO.

Considerando os efeitos cardíacos provenientes da atividade adrenérgica exacerbada, diversas modalidades de tratamento farmacológico foram propostas com o objetivo de prevenir ou reverter o fenótipo de remodelamento causado pela hiperatividade simpática. O antagonismo dos receptores β-adrenérgicos foi eficiente em prevenir (Ljubuncić et al, 1992a; Ljubuncić et al, 1992b) ou reverter (Brouri et al, 2004) a necrose e fibrose miocárdica induzidas pelo iso. Ljubuncić et al (1992c), ao tratarem preventivamente ratos com um bloqueador do canal de cálcio (verapamil), observaram completa proteção contra a necrose miocárdica induzida pelo ISO. Adicionalmente, o uso do bloqueador do canal de cálcio nifedipina mostrou reverter o estresse oxidativo causado pelo ISO e, quando adicionado à terapia com bloqueador do receptor AT_1 da angiotensina II, foi efetivo em aumentar a densidade capilar e preservar as propriedades

ultraestruturais do miocárdio (Okuda et al, 2005). Não obstante, a administração de antagonista dos receptores de mineralocorticóide e de substâncias antioxidantes (Ljubuncić et al, 1991; Gallego et al, 2001; Ishizawa et al, 2006; Liu et al, 2009) foi eficiente em reduzir a injúria celular e o acúmulo de colágeno no miocárdio, assim como em melhorar o metabolismo energético mitocondrial.

É amplamente aceito que o exercício físico (EXF) é uma intervenção valiosa para promoção de saúde, melhoria da qualidade de vida e prevenção e/ou controle de diversas patologias cardiovasculares (Pate et al, 1995; Franklin & Kahn, 1996; Mechelen, 1997; Hambrecht et al, 1999; Hambrecht et al, 2000; Fletcher et al., 2001; Paterick & Fletcher, 2001; Lee & Skerrett, 2001; Billman, 2002; Myers, 2003; Giannuzzi et al, 2003; Williams et al, 2007; Ascenção et al, 2007; Gibala, 2007; Peterson, 2007; Moholdt et al, 2008; Yap & Davis, 2008; Hoehner et al, 2008; Tanaka, 2009; Cornelissen et al, 2009; Prasad & Das, 2009). Como intervenção terapêutica, o EXF induziu efeitos cardíacos favoráveis em humanos e animais de laboratório com hipertensão arterial sistêmica (Kawamura et al., 2002; Bertagnolli et al., 2008; Cornelissen et al, 2009; Garciarena et al, 2009), infarto do miocárdio (Zhang et al, 2000a; Zhang et al, 2000b; Giannuzzi et al, 2003; Lennon et al, 2004; Freimann et al, 2005; Zanchi et al, 2008; Vona et al, 2009; Freimann et al, 2009) e insuficiência cardíaca (Belardinelli et al, 1999; Hambrecht et al, 2000; Corrà et al, 2003; Portes & Tucci, 2006; Bocalini et al, 2008; Owen et al, 2009).

Dados originários de ensaios clínicos deram outros esclarecimentos acerca do efeito cardioprotetor do EXF, sobretudo em pacientes com insuficiência cardíaca, nos quais houve abrandamento da atividade nervosa simpática exacerbada (Coats et al, 1992; Kiilavuori et al, 1999; Tyni-Lenné et al, 2001; Roveda et al, 2003; Passino et al, 2006;

Fraga et al, 2007; Negrão & Middlekauff, 2008). Contudo, tais estudos somente descreveram os efeitos benéficos do EXF na expressão de marcadores adrenérgicos como a noradrenalina ou a frequência de disparos dos nervos simpáticos. Além disso, a análise do papel protetor do EXF em ensaios clínicos inclui certos inconvenientes que podem dificultar o entendimento da matéria. Dificuldades peculiares são geradas pela diversidade da casuística em relação às faixas etárias, à intensidade e duração da estimulação adrenérgica, ao tempo de evolução e grau da insuficiência cardíaca e à associação com outras doenças e outros medicamentos. A ausência destes fatores de confusão nos ensaios com animais de laboratório, que permite controle mais acurado dos fatores intervenientes nos resultados, solidifica a importância dos estudos experimentais.

Em condições experimentais, achados convincentes reforçam o potencial salutar do EXF como estratégia não-farmacológica eficaz frente à estimulação adrenérgica cardíaca anômala. Neste sentido, diversos trabalhos analisaram a influência do exercício físico na injúria miocárdica de animais expostos à sobrecarga adrenérgica com ISO. O foco principal destes estudos foi avaliar o caráter preventivo do EXF em animais com necrose do miocárdio induzida por grandes doses de ISO, que variaram entre 20 a 250 mg/kg/dia.

Em estudo precursor, Tajuddin et al (1975) demonstraram que o EXF prévio ao ISO atenuou os níveis séricos da desidrogenase láctica (DHL) e creatinoquinase (CQ) de animais treinados em natação. Depois da divulgação destes dados, foi nítido o aumento do interesse acerca do papel cardioprotetor do EXF. Embora não façam menção a efeitos protetores do EXF na injúria cardíaca associada ao ISO, Riggs Jr et al. (1977) reportaram mortalidade reduzida em ratos previamente submetidos a regime de treinamento em

esteira rolante. Crandall et al. (1981) avaliaram a extensão da injúria miocárdica analisando a atividade plasmática da isoforma 1 da DHL em corações de ratos previamente treinados em esteira e verificaram atenuação do aumento da DHL provocada pelo ISO e inibição da mortalidade. Resultado equivalente, de redução da injúria miocárdica avaliada pela atividade da CQ no miocárdio, foi divulgado por Brodowicz & Lamb (1991), que estudaram os efeitos preventivos de 14 semanas de treinamento em esteira. Além destas particularidades, o EXF iniciado antes da administração de ISO foi eficaz em reduzir as anormalidades eletrocardiográficas sugestivas de necrose miocárdica (Darrah & Engen, 1982).

A revisão da literatura conduziu à conclusão de que, embora seja inegável a contribuição valiosa dos estudos iniciais acerca do papel cardioprotetor do EXF na sobrecarga adrenérgica, não há nenhum relato que tenha se preocupado, prioritariamente, em avaliar a influência do exercício físico prévio nas modificações estruturais, funcionais e moleculares cardíacas promovidas pelo ISO. Nesta perspectiva, este estudo foi conduzido para analisar o papel cardioprotetor do EXF em ratos tratados cronicamente com ISO. Como protocolo experimental nos valemos das recomendações de Tang & Taylor (1984a) que propõem a ministração de iso durante oito dias consecutivos, na dose de 0,3 mg/kg de peso corporal, por via subcutânea. Este protocolo induz HM com boa reprodutibilidade e baixa mortalidade (Taylor et al, 1977; Tang & Taylor, 1984a; Jalil et al, 1989; Tang & Taylor, 1984b). Com o intuito de promover cardioproteção, um programa de treinamento físico em esteira rolante, de intensidade moderada e duração de 13 semanas, foi instituído previamente ao ISO. Protocolos similares de EXF mostraram efeitos positivos em ratos com hipertensão arterial sistêmica (Kawamura et al., 2002;

Bertagnolli et al., 2008), injúria por isquemia-reperfusão (Lennon et al., 2004; Quindry et al., 2005; Le Page et al., 2009) e insuficiência cardíaca consequente de infarto do miocárdio (Batista et al., 2007; Zanchi et al., 2008).

Este texto reúne dois trabalhos que analisam a influência do EXF sobre as modificações moleculares, celulares, estruturais e funcionais do coração promovidas pela administração prolongada de ISO. Um deles foi divulgado no European Journal of Heart Failure (*artigo 1*) e o outro foi submetido à análise do periódico Basic Research in Cardiology (*artigo 2*).

OBJETIVO GERAL

Avaliar o possível efeito protetor cardíaco do exercício físico prévio em ratos submetidos a um modelo experimental de sobrecarga beta-adrenérgica com isoproterenol.

OBJETIVOS ESPECÍFICOS

Artigo 1

Avaliar o papel do exercício físico prévio nas modificações estruturais e ultraestruturais cardíacas desencadeadas pela sobrecarga adrenérgica com isoproterenol.

Artigo 2

Analisar os efeitos do exercício físico prévio na hipertrofia e na disfunção do miocárdio de ratos tratados com isoproterenol, procurando determinar possíveis mecanismos moleculares envolvidos na cardioproteção.

ARTIGO 1

O exercício físico previne a hipertrofia e injúria miocárdica induzidas por sobrecarga beta-adrenérgica

Exercise training prevents β-adrenergic hyperactivity-induced myocardial hypertrophy and lesions

Exercise training prevents β-adrenergic hyperactivity-induced myocardial hypertrophy and lesions

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Abstract

Background: Sustained β-adrenoreceptor activation promotes cardiac hypertrophy and cellular injury.

Aims: To evaluate the cardioprotective effect of exercise on damage induced by β-adrenergic hyperactivity.

Methods: Male Wistar rats were randomised into four groups (n = 8 per group): sedentary non-treated control (C), sedentary treated with isoproterenol 0.3 mg/kg/day administered subcutaneously for 8 days (I), exercised non-treated (E) and exercised plus isoproterenol administered during the last eight days of exercise (IE). Exercised animals ran on a treadmill for 1 h daily 6 times a week for 13 weeks.

Results: Isoproterenol caused increases in left ventricle (LV) wet and dry weight/body weight ratio, LV water content and cardiomyocyte transverse diameter. Additionally, isoproterenol induced severe cellular lesions, necrosis, and apoptosis, increased collagen content and reduced capillary and fibre fractional areas. Notably, all of these abnormalities were completely prevented by exercise.

Conclusion: Our data have demonstrated that complete cardioprotection is possible through exercise training; by preventing β-adrenergic hyperactivity-induced cardiac hypertrophy and structural injury.

Key words: Myocardial hypertrophy, Isoproterenol, Exercise training, Cardioprotection.

1. INTRODUCTION

Pronounced activation of the sympathetic nervous system in heart failure (HF) is inversely correlated with survival [1] and the clinical use of beta-adrenoreceptor antagonists is now recommended as a part of the gold-standard treatment for heart failure [2]. Accordingly, experimental models of adrenergic hyperactivity have gained interest. Isoproterenol (ISO), a synthetic non-selective β-adrenergic agonist, causes severe cardiac injury by promoting myocardial hypertrophy [3] and a necrosis-like infarction [4] of the heart muscle through inflammation [5], cytosolic Ca2+ overload [3,6], and oxygen-derived free radicals generation [7]. Hence, several treatment schemes with potential for preventing the structural myocardial modifications induced by the β-adrenergic actions of

ISO have been evaluated: including calcium channel blockers [8], angiotensin-converting enzyme inhibitors [9], AT1 angiotensin II receptors blockers [8], β-adrenergic receptor antagonists [10], and antioxidants [11].

Although the cardioprotective effects of exercise training have been extensively described [12,13], only a few studies have investigated the possible benefits of exercise training prior to ISO administration. Riggs et al. [14], and Brodowicz and Lamb [15] studied serum and myocardial creatine kinase activity 24 h after a single injection of isoproterenol. Darrah et al. [16] examined the electrocardiographic changes associated with myocardial necrosis induced by two subcutaneous injections of ISO. These authors reported that exercise only attenuates myocardial lesions caused by very large doses of ISO.

In spite of the known beneficial cardiovascular effects of exercise, there is no data on myocardial structural characteristics in animals undergoing exercise training prior to β-adrenergic hyperactivity induced by ISO. The aim of this study was therefore to describe the structural myocardial changes following ISO administration, and to evaluate the effect of previous exercise training on these changes. A more detailed morphological characterization of ISO-induced myocardial lesions in animal models, and an evaluation of the effect of exercise training preceding ISO administration, may help in the understanding of HF pathogenesis in terms of β-adrenergic receptor hyperactivity and its treatment.

2. MATERIAL AND METHODS

2.1. Animals and administration of ISO

Thirty-two male Wistar rats weighing 150-180 g were randomly assigned to four treatment groups (n = 8 per group): 1) non-trained control rats that received only olive oil (Control group: C); 2) non-trained rats that received subcutaneous injections of ISO (0.3 mg/kg/day) diluted in 1 ml of olive oil (ISO group: I); 3) trained rats that received only olive oil (exercise group: E); and 4) trained rats that received ISO injections (ISO plus E group: IE). Animals were cared for in compliance with the "Principles of Laboratory Animal Care" formulated by the National Institutes of Health (National Institutes of Health publication no. 96-23, revised, 1996), according to a

protocol approved by the Ethics Research Committee at the Federal University of São Paulo, Brazil.

2.2. Exercise protocol

Animals were made to run on a treadmill for 1 h per day, 6 days per week. This exercise protocol lasted for 13 weeks. The treadmill speed was set at 18 m/min for the first 30 min and was increased to 22 m/min for the remaining 30 min of exercise. Before the start of the formal exercise protocol, rats were preconditioned to treadmill running for 12 consecutive days; during this time the treadmill speed was progressively increased by 3 m/min every 2 days until the final speed of 18 m/min was reached. The sessions initially lasted for 5 min and were increased by 5 min each day to reach 60 min on day 12. ISO or olive oil was administered on the last day of week 12 and on all seven days of week 13 of the exercise protocol, to achieve 8 days of treatment. Twenty-four hours after the last training session the rats were anesthetized (urethane: 1.2 g/kg) and sacrificed. Eight rats in each group were studied for evaluation of myocardial mass and LV water content. Left ventricular wet weight (WW) and dry weight (DW) were determined before and after samples were dried at 70 °C until they achieved constant weight in order to determine myocardial water content (H_2O), which was estimated using the formula:

$$H_2O\ (\%) = [(WW - DW)/WW] \times 100$$

2.3. Histomorphometric analysis

After anesthesia, the right carotid artery was cannulated and 1 ml of KCL 19% was injected to promote cardiac arrest. The thorax was opened, the aorta was occluded just beyond the right carotid artery and the heart was removed. Thereafter, the right atrium was opened and the heart was perfused with phosphate buffer (0.01 mM; pH 7.4) for 2 min, and then with 10% formalin buffered solution with 2% glutaraldehyde for another 10 min under 90 mmHg of perfusion pressure. The heart was transversally sectioned at the mid-ventricular level; the basal portion was utilized for electron microscopy studies and the apical portion was used for optical microscopic examination. Small LV fragments were sampled for electron microscopy study, after fixing in 2% glutaraldehyde at pH 7.2. The apical portion was fixed in a mixture of buffered formalin 10% and glutaraldehyde 2% and processed for

embedding in paraffin and optical microscopic examination.

For optical microscopic examination, 7 μm thickness sections were obtained from the left ventricular equator and haematoxylin-eosin stained. LV diameter and free wall thickness were determined in these transverse cardiac sections. Nuclear length (major diameter) and width (minor diameter) were then measured. Fifty nuclei from each animal (n = 3 per group) were measured. The nuclear volume (V) was estimated from the formula for a prolate ellipsoid [17]: $V = \pi AB2 / 6$; where A is the major diameter and B is the minor diameter. Collagen content was determined by picrosirius red staining of sections of myocardium and analyzed using polarized light observation [18]. Histological images were visualized using an Olympus microscope at 40× magnification, and analyzed using Image Tool software 3.0.

Ultrastructural evaluation was performed in three rats from each group by electron microscopy. Fragments were cut into small 1 mm thick pieces, post-fixed in 1% OsO_4 solution for 2 h at 4 °C, and then dehydrated and embedded in araldite. Silver or grey thin sections were cut on a Porter-Blum MT-B ultra microtome, mounted on copper grids and stained with uranyl acetate and lead citrate. Preparations were examined through a Philips EM-301 electron microscope and photographed at 1.650× or 8.900× the original magnification. For each rat, cardiomyocyte transverse diameter was measured at the nucleus level in five longitudinally oriented cells. A 1.056 point-reticule was superimposed on five representative microphotographs from each rat; and capillary and fibre fractional areas were calculated according to the method described by Gundersen et al. [19]. Means of the five measurements were considered representative for each rat.

2.4. TUNEL staining

In order to detect apoptotic cells, a fluorescent TUNEL assay was performed in 2-cm long, 5-μm thick paraffin embedded, formalin-fixed myocardial sections from the medial poles of the resected fragments. In brief, sections were dewaxed in xylene and rehydrated. Sections were pretreated with proteinase K (Gibco, Gaithensburg, MD) at a dilution of 20 μg mL^{-1} in 10 mM Tris/HCL, pH 7.5 for 15 min at 32-35 °C. Sections were rinsed in 0 1 M

phosphate buffered saline (PBS), pH 7.3, and incubated in a humid atmosphere at 37 °C for 60 min in 50 μL of TUNEL reaction mixture coupled with fluorescein (Boehringer, Mannheim, Germany). Positive-stained controls were prepared through incubation of serial sections of each paraffin block with 10 U/ml DNase I for 20 min at 37 °C before treatment with terminal transferase. Negative controls were prepared by staining serial slides without terminal deoxynucleotide transferase. The number of TUNEL-positive myocardial cells was counted using 200× microscopic enlargement in 5 randomly chosen fields (1 mm^2) of each rat and expressed as the number of apoptotic cells/10.000 cardiomyocytes. The actual area of myocardium studied was measured using a Leica Q Win (Leica, Bensheim, Germany) image analysis system coupled with a Zeis SV6 stereomicroscope. The mean area studied was 179±34 mm^2. The cardiomyocyte origin of the TUNEL-labelled cells was confirmed by staining of muscular elements with phalloidin conjugated to TRITC. For this purpose, TUNEL-stained sections were incubated for 40 min with a TRITC conjugated phalloidin solution (Sigma) at a dilution of 1 mg mL^{-1} in PBS containing 0.1% Triton-X-100. Double-stained sections were analyzed by confocal microscopy using a Noran confocal microscope.

2.5. Statistical analysis

One way ANOVA complemented by Newman–Keuls test was used to detect differences between groups. A p value < 0.05 was considered significant and results are presented as means ± standard deviation (SD).

3. RESULTS

3.1. Myocardial mass

Results are summarized in Table 1. Non-trained ISO-treated rats (Group I) had significantly higher values of heart weight/body weight, LV wet weight/body weight and LV dry weight/body weight compared to the other groups. The tissue weight increase was accompanied by oedema as LV water content was significantly higher. Exercise did not affect myocardial mass or water content. Exercise combined with ISO prevented the changes in mass and water content observed in Group I.

3.2. Optical microscopy

Non-trained ISO-treated rats (Group I)

Table 1. Structural data (mean ± SD) for control rats (C), non-trained rats that received ISO (I), trained rats that received only olive oil (E) and trained rats that received ISO injections (IE).

Data	Experimental groups				P value
	C	I	E	IE	
HW/BW (mg/g)	2.3 ± 0.1^a	3.4 ± 0.4^b	2.6 ± 0.4^a	2.7 ± 0.2^a	< 0.001
LVWW/BW (mg/g)	1.7 ± 0.1^a	2.6 ± 0.3^b	2.0 ± 0.4^a	2.0 ± 0.2^a	<0.001
LVDW/BW (mg/g)	0.48 ± 0.08^a	0.60 ± 0.05^b	0.52 ± 0.08^a	0.50 ± 0.05^a	<0.05
H_2O (%)	75.0 ± 1.3^a	77.5 ± 1.1^b	75.8 ± 0.6^a	75.6 ± 0.6^a	<0.01
LVCD (mm)	6.6 ± 0.4^a	5.3 ± 0.3^b	6.8 ± 0.5^a	6.7 ± 0.5^a	<0.001
FWT (mm)	1.26 ± 0.09^a	1.88 ± 0.08^b	1.23 ± 0.13^a	1.23 ± 0.14^a	<0.001
V (μm^3)	125 ± 22^a	229 ± 10^b	127 ± 13^a	121 ± 13^a	<0.001
CD (μm)	12 ± 1^a	21 ± 2^b	12 ± 1^a	12 ± 1^a	<0.001
CFA (%)	6.0 ± 2.0^a	2.4 ± 1.2^b	5.8 ± 0.9^a	6.0 ± 0.4^a	<0.05
FFA (%)	60 ± 4^a	44 ± 7^b	65 ± 1^a	59 ± 8^a	<0.05

HW/BW: heart weight/body weight (n = 8), LVWW/BW: left ventricular wet weight/body weight (n = 8); LVDW/BW: LV dry weight/body weight (n = 8); H_2O: LV water content (n=8); LVCD: LV chamber diameter (n = 8); FWT: LV free wall thickness (n = 8); V: nuclear volume (n = 8); CD: cardiomyocytes transverse diameter (n = 3); CFA: capillary fractional area (n = 3); FFA: fibres fractional area (n = 3). Same letters indicate values not different in ANOVA. Different letters indicate significant difference between means.

had significantly smaller LV chamber diameter and increased free wall thickness (Table 1) characterizing the occurrence of LV concentric hypertrophy, which corroborates previous findings in our lab [20]. The ISO-treated rats showed significantly increased nuclear volume in LV (Table 1), multiple foci of subendocardial necrosis, subendocardial fibrosis, and increased myocardial collagen. Subendocardial necrosis foci were represented by granulation tissue with lymphomononuclear inflammatory infiltrate. As expected, these changes were not present in Groups C or E, and importantly, the rats in Group IE had a normal histological appearance (Fig. 1).

3.3. Transmission electronic microscopy

Electron microscopy (Fig. 2) showed that Group I had important changes in myocardial fibres, as represented by myofilament lysis, sarcolemmal rupture and intracellular and extracellular oedema. Histomorphometric analysis (Table 1) of sedentary treated rats

revealed significantly larger cardiomyocyte diameter, and reduction in capillary and myocardial fibre fractional areas. In all evaluations, the cardioprotective effect of exercise was characterized. Indeed, ultra structural evaluation of the myocardium of ISO trained rats (Group IE) was essentially normal, as was the case in Group C and Group E rats.

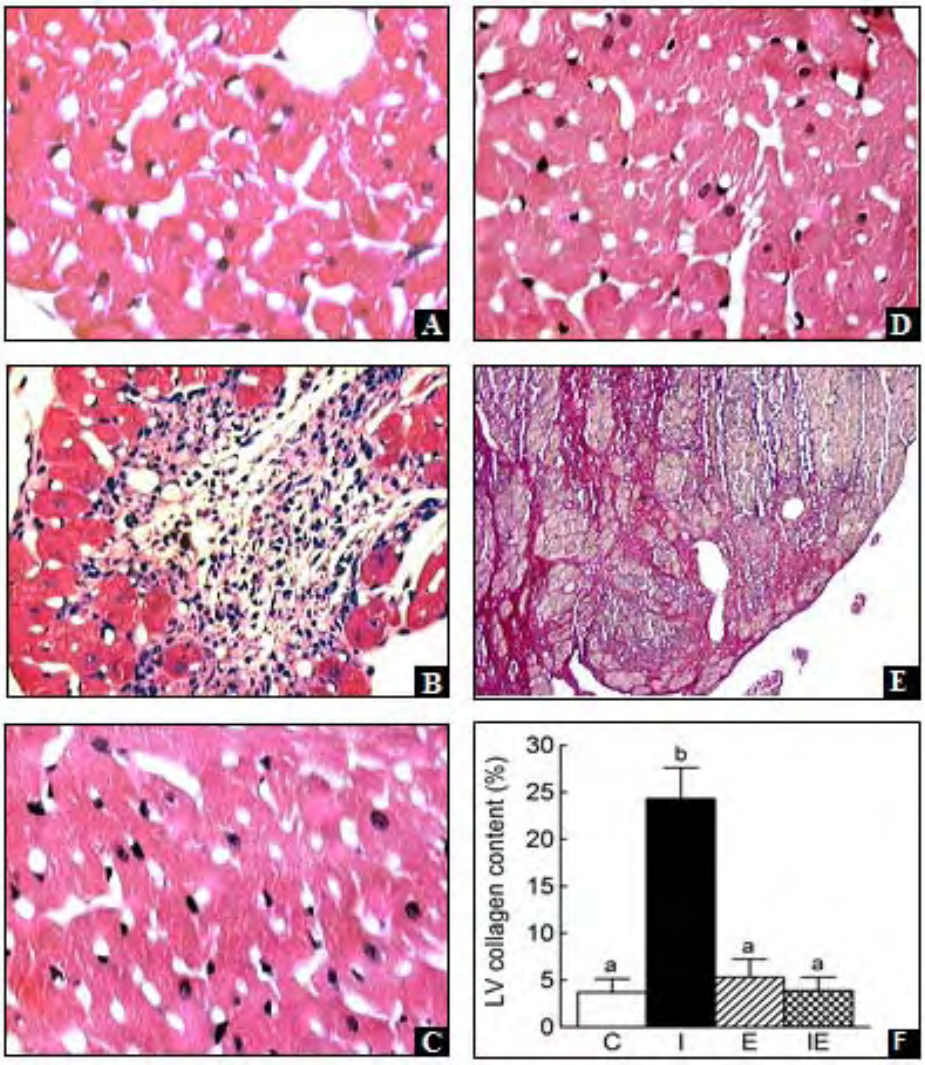

Figure 1. Optical micrographs (n = 8 per group). Panel (A-D): haematoxylin-eosin staining. Panel (E) picrosirius red. Panels A, C and D: show normal morphology of the LV myocardium in groups C, E and IE, respectively. Panel B: Granulation tissue, lymphomononuclear inflammatory infiltrate and diffuse myocardial necrosis, as well as subendocardial fibrosis (panel E) and increase in collagen content (Panel F) were seen in group I. All changes were suppressed by exercise training. Same letters indicate values not different in ANOVA. Different letters indicate significant difference between means.

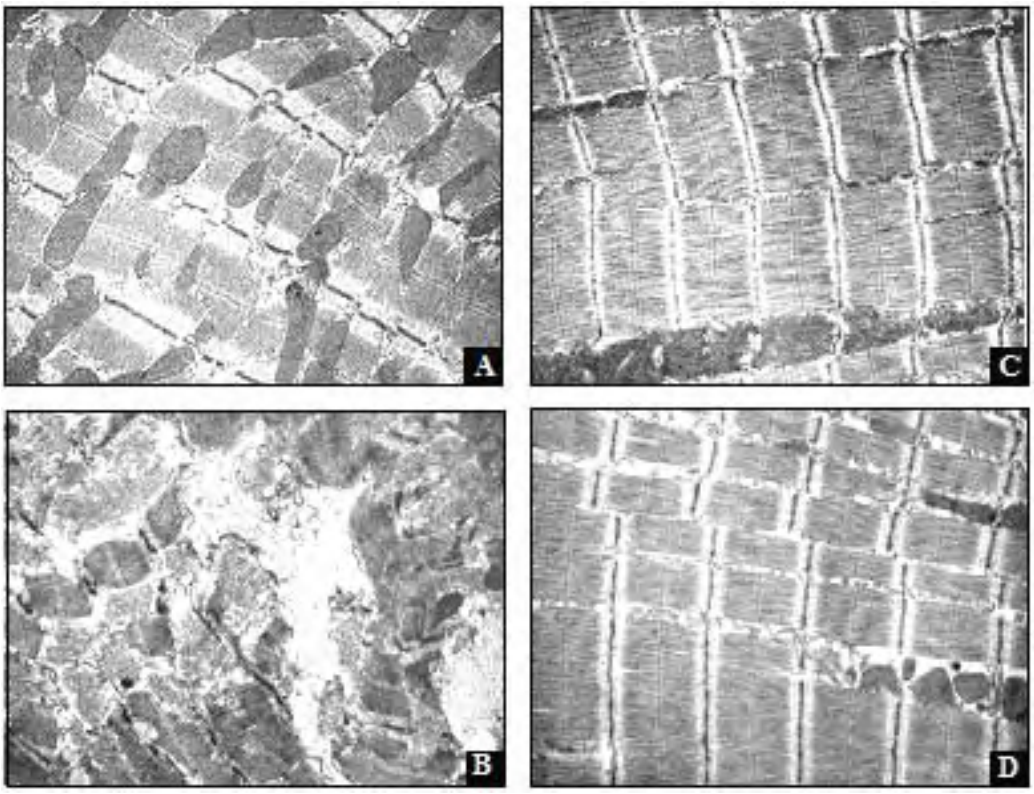

Figure 2. Electron micrographs (magnification: 8900×; n = 3 per group). Group C (Panel A) and Group E (Panel C) rats showed normal myocardium. ISO rats (Panel: B) showed severe cellular injury, including myofilament lysis, sarcolemmal rupture, and intracellular and extracellular oedema. Exercise trained ISO rats (Panel: D) showed totally preserved ultrastructure.

3.4. TUNEL staining

ISO treatment significantly increased (p=0.01) the number of apoptotic cells (C: 15.0±8.6; I: 46.1±24.5; E: 12.5±10.0; IE: 9.7±5.4) normalized per 10.000 cardiomyocytes (Fig. 3). This increase in apoptotic cardiomyocytes was prevented by exercise training.

4. DISCUSSION

Our data showed that non-trained ISO animals had heavier hearts, higher LV dry weight, LV water content and cardiomyocyte transverse diameter when compared to the control group; indicating that ISO caused myocardial oedema, increased protein content and cardiomyocyte hypertrophy. Since nuclear enlargement is associated with cellular hypertrophy [17], cardiomyocyte growth was confirmed by the increase in nuclear volume. Additionally, our results on myocardial mass, LV chamber transverse diameter and free wall

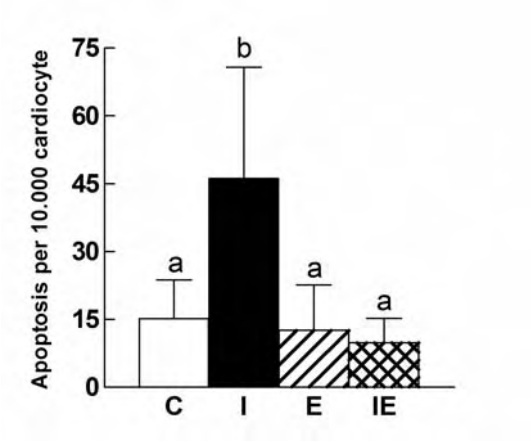

Figure 3. Fig. 3. Apoptotic cardiomyocytes in experimental animals (n = 8 per group) estimated from positive control slides of serial sections (per 10.000 cardiomyocyte nuclei). Same letters indicate values not different in ANOVA. Different letters indicate significant difference between means.

thickness, indicate that ISO-induced cardiac hypertrophy was of the concentric type, which corroborates previous findings in our lab [20]. Taking into account the myofilament lysis in association with the sarcolemmal rupture, indicating ISO-induced myocardial necrosis, it can be proposed that collagen content increase (Fig. 1F) occurs as reparative fibrosis [21]. In addition, apoptosis has been shown to coexist with necrosis in the heart following ISO, and increases cardiomyocyte loss. Marked myocardial necrosis and apoptosis, coexisting with increased collagen content in these animals, explains the impaired ability of the myocardium to generate force in combination with more marked elastic stiffness, as described previously [9,20]. Chronic β-adrenergic activation is thought [3] to promote cardiomyocyte hypertrophy via local stimulation of myocardial growth factors such as angiotensin II, transforming growth factor β_1 and insulin-like growth factor-1. The pathogenesis of catecholamine-induced myocardial injury has yet to be fully defined. Mechanisms previously proposed [11,22–24] include: ischaemia-reperfusion injury, free radical generation, cAMP-dependent calcium overload of cardiac myocytes, and activation of the renin–angiotensin–aldosterone system. It can be supposed that the significant reduction in myocardial capillary fractional area in sedentary ISO-treated rats could cause reduced oxygen delivery to cardiomyocytes due to capillary deficiency, thus contributing to myocardial necrosis and apoptosis in these animals. In contrast, in the IE rats, the exercise regime was able to inhibit the reduction in capillary fractional area, which may have maintained oxygen delivery to the cardiomyocytes during ISO administration in the exercised rats,

thus preventing tissue ischaemia.

Calcium channel blockers [8], angiotensin-converting enzyme inhibitors [9], AT_1 angiotensin II receptors blockers [8], β-adrenergic receptors antagonists [10], and antioxidants [11] have been shown to prevent the cardiac hypertrophy and structural injury caused by β-adrenergic agonists. In our study, we demonstrated complete protection from cardiac hypertrophy and myocardial lesions in rats that received ISO after exercise training. To the best of our knowledge, this is the first demonstration of full protection against cardiac lesions secondary to β-adrenergic stimulation by exercise training. Previous studies have shown that exercise training only attenuates ISO-induced myocardial necrosis [14–16], rather than completely abolishing the lesions as occurred in our study. It is possible that the very high doses of ISO used in these previous studies (20 mg/kg, 70 mg/kg and 250 mg/kg, respectively) may have made full cardioprotection impossible. Indeed, taking into account the fact that the cardiac effects observed at the dose used in our study were clearly toxicological and never having been seen in physiological β-adrenergic stimulation, we consider that the amount of ISO used by these authors is clearly non-physiological. This is particularly important because β-adrenergic stimulation is known [25] to cause dose-dependent cardiac injury. In light of these facts, we conclude that these previous studies were unlikely to demonstrate exercise induced cardioprotection against physiological β-adrenergic hyperactivity. In addition, assuming that the ISO dose used in our study is also excessive in terms of usual physiological β-adrenergic stimulation, for clinical purposes, exercise training can be considered as very effective in promoting heart protection against β-adrenergic hyperactivity.

In conclusion, the cardioprotective effect of exercise against lesions due to β-adrenergic stimulation was complete: that is to say it included prevention of myocardial hypertrophy, myocardial necrosis and apoptosis, inflammation, cardiac oedema, subendocardial fibrosis, increased collagen content, and reduction in myocardial fibre and capillary fractional area. These results strongly suggest that regular exercise is a very effective non-pharmacological intervention for protecting the heart

against β-adrenergic injury.

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5. REFERENCES

1. Packer M. Neurohormonal interactions and adaptations in congestive heart failure. Circulation 1988;77:721-30.

2. Hunt SA, Abraham WT, Chin MH, et al. ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). American College of Cardiology Web Site. Available at: http://www.acc.org/clinical/guidelines/failure//index.pdf. Circulation 2005; 112:154-235.

3. Osadchii OE. Cardiac hypertrophy induced by sustained β-adrenoreceptor activation: pathophysiological aspects. Heart Fail Rev 2007;12:66-86.

4. Prabhu S, Jainu M, Sabitha KE. Effect of mangiferin on mitochondrial energy production in experimentally induced myocardial infarcted rats. Vascul Pharmacol 2006;44:519-41.

5. Murray DR, Prabhu SD, Chandrasekar B. Chronic beta-adrenergic stimulation induces myocardial proinflammatory cytokine expression. Circulation 2000;101:2338-41.

6. Rona G, Chappel CI, Balazs T, Gaudry R. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. AMA Arch Pathol 1959;67:443-55.

7. Rathore N, John S, Kale M, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. Pharmacol Res 1998,38:297-303.

8. Okuda N, Hayashi T, Mori T, et al. Nifedipine enhances the cardioprotective effect of angiotensin-II receptor blocker in an experimental animal model of heart failure. Hypertens Res 2005;28:431-38.

9. Gallego M, Espina L, Vegas L. Spironolactone and captopril attenuates isoproterenol-induced cardiac remodelling in rats. Pharmacol Res 2001;44:311-5.

10. Brouri F, Hanoun N, Mediani O, et

al. Blockade of β1- and desensitization of β2-adrenoreceptors reduce isoprenaline-induced cardiac fibrosis. Eur J Pharmacol 2004;485:227-34.

11. Ishizawa M, Mizushige K, Noma T, et al. An antioxidant treatment potentially protects myocardial energy metabolism by regulating uncoupling protein 2 expression in a chronic β-adrenergic stimulation rat model. Life Sci 2006;78:2974-82.

12. Lennon SL, Quindry JC, French JP, Kim S, Mehta JL, Powers SK. Exercise and myocardial tolerance to ischaemia-reperfusion. Acta Physiol Scand 2004;182: 161-9.

13. Freimann S, Scheinowitz M, Yekutieli D, Feinberg MS, Eldar M, Kessler IG. Prior exercise training improves the outcome of acute myocardial infarction in the rat: heart structure, function, and gene expression. J Am Coll Cardiol 2005;45:931-8.

14. Riggs CE, Landiss CW, Jessup GT, Bonner HW. Effects of exercise on the severity of isoproterenol-induced myocardial infarction. Med Sci Sports 1977;9:83-7.

15. Brodowicz GR, Lamb DR. Exercise training, indomethacin, and isoproterenol-induced myocardial necrosis in the rat. Basic Res Cardiol 1991;86:40-8.

16. Darrah M, Engen RL. Beneficial effects of exercise on *l*-isoproterenol induced myocardial infarction in male rats. Med Sci Sports Exerc 1982;14:76-80.

17. Gerdes AM, Liu Z, Zimmer HG. Changes in nuclear size of cardiac myocytes during the development and progression of hypertrophy in rats. Cardioscience 1994;5:203-8.

18. Junqueira LCU, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochem J 1979;11:442-55.

19. Gundersen HJG, Bendtsen TF, Korbo L, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988;96:379-94.

20. Murad N, Tucci PJ. Isoproterenol-induced hypertrophy may result in distinct ventricular changes. Clin Exp Pharmacol Physiol 2000;27:352-7.

21. Benjamin IJ, Jalil JE, Tan LB, et al. Isoproterenol-induced myocardial fibrosis in relation to myocite necrosis. Circ Res 1989;65: 657-70.

22. Mann DL. Basic mechanisms of

disease progression in the failing heart: the role of excessive adrenergic drive. Prog Cardiovasc Dis 1998;41(suppl 1):1-8.

23. Grimm D, Elsner D, Schunkert H, et al. Development of heart failure following isoproterenol administration in the rat: role of the renin-angiotensin system. Cardiovasc Res1998;37:91-100.

24. Zhou B, Wu L-J, Li L-H, Tashiro S-I, Onodera S, Uchiumi F, IkejimaT. Silibini protects against isoproterenol-induced rat cardiac myocyte injury through mitochondrial pathway after up-regulation of SIRT1. J Pharmacol Sci 2006;102:387-95.

25. Teerlink JR, Pfeffer MA. Progressive ventricular remodeling in response to diffuse isoproterenol-induced myocardial necrosis in rats. Circ Res 1994;75:105-13.

ARTIGO 2

O exercício físico previne a hipertrofia e disfunção do miocárdio e melhora o inotropismo em ratos com hiperatividade β-adrenérgica sustentada

Exercise training prevents myocardial hypertrophy and dysfunction and improves inotropism in rat with sustained β-adrenergic hyperactivity

Exercise training prevents myocardial hypertrophy and dysfunction and improves inotropism in rat with sustained β-adrenergic hyperactivity

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Abstract

To test the hypothesis that exercise training can prevent the myocardial dysfunction and to inhibit the left ventricular (LV) remodeling induced by β-adrenergic hyperactivity, Wistar male rats were assigned to four groups: sedentary non-treated (CON), sedentary isoproterenol- treated (ISO), exercised non-treated (EX), and exercised plus isoproterenol (I+E). Echocardiography, hemodynamic and isolated papillary muscle were used for functional evaluations. Real time RT-PCR and western blot were used to quantify TNF-α, IL-6, IL-10 and TGF-β_1 in the tissue. The nuclear NF-κB expression was evaluated by immunohistochemical staining. The ISO rats showed a concentric hypertrophy of LV. These animals exhibited marked increases in LV end-diastolic pressure and impaired myocardial performance *in vitro* by reducing the developed tension and maximum rate of tension increase and decrease, as well as worsened recruitment of the Frank-Starling mechanism. Both gene and protein levels of TNF-α and IL-6 as well as the TGF-β_1 mRNA were also increased. In addition, the nuclear NF-kB expression in the ISO group was significantly raised. In the I+E group, the exercise training (i) prevented LV hypertrophy, (ii) improved myocardial contractility, (iii) avoided the increase of pro-inflammatory cytokines and improved IL-10 levels, and (iiii) attenuated the increase of TGF-β_1 mRNA. Thus, exercise training in a model of β-adrenergic hyperactivity can avoid the adverse remodeling of LV and improve the myocardial contractile. It appears likely that the cardioprotection is related to beneficial effects of exercise on balance between pro- and anti-inflammatory cytokines, and mitigates the expressions of TGF-β_1.

Key words: Exercise training, Cardioprotection, Hypertrophy, β-adrenergic hyperactivity, Isoproterenol, Myocardial dysfunction, Cytokines, growth factors.

1. INTRODUCTION

It has been known that left ventricular hypertrophy is an independent and powerful predictor of heart failure and mortality [1,2]. There is a growing body of evidence showing that heart failure is often associated with an increase in intracardiac sympathetic nerve activity [3,4], which intensifies myocardial remodeling [5-8].

Considering the inconvenience of sustained β-adrenergic cardiac stimulation, much effort is currently being undertaken to find novel treatment modalities to prevent or even reverse the remodeling phenotype induced by β-adrenergic overload. Several potential pharmacological schemes for preventing myocardial remodeling of β-adrenergic-induced injury have been previously evaluated: β-adrenergic receptor antagonists [9], angiotensin-converting enzyme inhibitors [10], calcium channel blockers [11], AT_1 angiotensin II receptor blockers [11], and antioxidants [12]. Additionally, information has been published regarding the cardioprotective effects of exercise training prior to β-adrenergic hyperactivity [13-15]. These studies have reported that exercise training only attenuates myocardial remodeling caused by very large doses of isoproterenol.

We have recently confirmed that, in rats, repeated injections of isoproterenol stimulate rapid hypertrophic growth [8]. In addition to myocardial hypertrophy, these rats develop necrosis, apoptosis, fibrosis, myocardial edema, and reduced capillary density. Furthermore, we demonstrated that exercise training completely prevents myocardial injury [8]. Nevertheless, we did not analyze the myocardial function and possible mechanisms by which the exercise training induced cardioprotection in these animals. Therefore, the purpose of this study was to examine the role of exercise training on the myocardium of rats submitted to β-adrenergic hyperactivity focusing: a) both hypertrophy and dysfunction; b) the myocardial expression of pro- and anti-inflammatory cytokines; c) the effects on transforming growth factor beta-1 and nuclear factor-kB expression.

2. MATERIAL AND METHODS

2.1. Animals and isoproterenol administration

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The protocol was approved by the Institutional Research Ethics Committee of the Federal University of São Paulo, Brazil. Forty-one male Wistar rats (160-190 g) were randomly assigned to one of four groups: 1) Con (n = 12), non-trained rats that received only vehicle (olive oil:

1 ml subcutaneously [sc]); 2) Iso (n = 13), non-trained rats that received isoproterenol sc injections (0.3 mg/kg/day) diluted in 1 ml of olive oil; 3) Ex (n = 8), exercise-trained rats that received only vehicle; and 4) Iso+Ex (n = 8), exercise-trained rats that received isoproterenol injections (0.3 mg/kg/day, sc) diluted in vehicle.

2.2. Exercise protocol

Rats were subjected to run on a motor-driven treadmill (CL – 4002; Caloi, São Paulo, Brazil) for 13 weeks, as previously reported [8]. Rats ran 6 times a week, and each session lasted up to 60 minutes. For each session, the speed race was 18 m/min for 30 min and 22 m/min for the remaining 30 min. Isoproterenol or olive oil were administered on the last day of week 12 and during the 7 days of week 13, thus covering 8 treatment days. Twenty-four hours after the last exercise session, the rats were anesthetized (urethane, 1.2 g/kg) and subjected to transthoracic echocardiography and left ventricle (LV) catheterization.

2.3. Echocardiography

As previously described [16], transthoracic echocardiography was performed using an HP Sonos-5500 (Hewlett Packard, Andover, MA, USA) echocardiograph with a 12-MHz linear transducer. Rats were imaged in the left lateral decubitus position with three electrodes placed on paws for the electrocardiogram. Two-dimensional paraesternal long- and short-axis views were recorded, as was 2D targeted M-mode tracings throughout the anterior and posterior left ventricular walls. The diastolic LV posterior wall thickness (LVPWd), systolic LV posterior wall thickness (LVPWs), LV end-diastolic (LVEDD), and LV end-systolic (LVESD) diameters, and LV fractional shortening (FS) were determined. Because the high heart rates caused fusion of the A and E waves, diastolic function was not evaluated by Doppler.

2.4. LV hemodynamics

Immediately after echocardiography, the rats were intubated, ventilated (rodent ventilator, Harvard Apparatus Mod 683; Holliston, MA, USA) and a 2-F Millar catheter-tip micromanometer was inserted through the right carotid artery into the LV cavity. Measurements of LV parameters, including LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and maxima positive (+dP/dt) and negative (–dP/dt) time derivatives of the developed pressure

were studied using AcqKnowledge 3.5.7 software (Biopac Systems Inc., Santa Barbara, CA, USA).

2.5. Myocardial mechanics

After hemodynamic study, the hearts were quickly removed and the posterior papillary muscles were placed in a tissue bath containing modified Krebs-Henseleit solution (in mM: 130 NaCl, 5.0 KCl, 1.2 $MgCl_2$, 1.5 $CaCl_2$ 1.5, 11 glucose, 20 U insulin, and 20 Hepes, 100% O_2, 29^o C, pH 7.4). Myocardial mechanics were evaluated as previously described [16]. Preparations were stimulated 12 times/min with 5 msecsquare-wave pulses through parallel platinum electrodes at voltages that were approximately 10% greater than the minimum stimulus required to produce a maximal mechanical response.

After a 60 min equilibration period, during which preparations were permitted to contract isotonically under light loading conditions (0.4 g), papillary muscles were loaded to contract isometrically for 15 min and stretched to the apices of their length–tension curves (L_{max}). The parameters were recorded through the use of AcqKnowledge 3.5.7 (Biopac Systems Inc.) software for later determination of peak developed tension (DT), maximum rate of tension increase (+dT/dt) and decrease (–dT/dt), and resting tension (RT). The developed and resting tension-length curves were derived from data obtained at lengths corresponding to 92%, 94%, 96%, 98%, and 100% of the L_{max}. At the end of the experiment, the muscle length at L_{max} was measured, and the muscular portion between the two clips was blotted dry and weighed. The cross-sectional area (CSA) was estimated from the muscle weight and length by assuming a cylindrical shape and a specific gravity of 1.0. All force-related data were normalized for the CSA.

2.6. Quantitative real time RT-PCR analysis

Total RNA in LV samples was extracted with TRIzol (Gibco BRL, Gaithersburg, MD, USA). The RNA was subjected to DNase digestion (Invitrogen). Reverse transcripition (RT) reaction was performed using a random primers with 200 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen). Real-time PCR was done with an Mastercycler ep Realplex (Eppendorf, Hamburg, Germany) using a SYBRGreen core reaction kit (Applied Biosystems, Foster City, CA, USA). The

primers used were as follows: rat TNF-α 195-305 (GenBank™ accession number X66539) forward primer 5'-AAATGGGCTCCCTCTATCAGTTC-3' and reverse primer 5'-TCTGCTTGGTGGTTTGCTACGAC-3'; rat IL-6 (GenBank™ accession number E02522) forward primer 5'-TCCTACCCCAACTTCCAATGCTC-3' and reverse primer 5'-TTGGATGGTCTTGGTCCTTAGCC-3'; rat IL-10 (GenBank™ accession number NM012854), forward primer 5'-AAAGCAAGGCAGTGGAGCAG-3' and reverse primer 5'-TCAAACTCATTCATGGCCTTGT-3'; rat TGF-β_1 (GenBank™ accession number 021578), forward primer 5′-TGGCGTTACCTTGGTAACC-3 and reverse primer 5′-GGTGTTGAG CCCTTTCCAG-3′; rat GAPDH (GenBank™ accession number NM017008) forward primer 5'-TGCACCACCAACTGCTTAGC-3' and reverse primer 5'-GCCCCACGGCCATCA-3'. One microliter of RT reaction was utilized for real-time PCR. Δ-ΔC_t values were normalized with the values obtained for amplification of GAPDH.

2.7. Western blot analysis

The frozen left ventricle was homogenized in cell lysis buffer (100 mM Tris, 50 mM NaCl, 10 mM EDTA, 1% Triton X-100) and a proteinase inhibitor cocktail (Sigma Chemical Corporation, St. Louis, MO, USA). Samples containing 30 μg of the homogenate were subjected to SDS-PAGE in 10% polyacrylamide gels. Separated proteins were transferred onto PVDF membranes (Hybond-P, Amersham Biosciences; Piscataway, NJ) and the transfer efficiency was monitored with 0.5% Ponceau S staining of the blot membrane. The membrane was soaked in a blocking buffer (5% nonfat dry milk, 10 mM Tris·HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween 20) for 2 h at room temperature and then incubated with rabbit anti-rat TNF-α (1:500; IBL-America, Minneapolis, MN, USA), goat anti-rat IL-6 (1:500; Abcam, Cambridge, MA,USA), and goat anti-rat IL-10 (1:200; R&D system, Minneapolis, MN, USA). After incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies, detection was performed with enhanced chemiluminescence reagents (Amersham Biosciences; Piscataway, NJ). The GAPDH expression levels were used to normalize the results.

2.8. Immunohistochemistry assay

Five-micron thick paraffin sections were deparaffinized and fixed in 10% buffered formaldehyde, and endogenous peroxidase activity was inactivated with 3% H_2O_2 for 15 min. The rabbit anti-rat against TNF-α (1:800) and NF-κB (1:2000) was used as primary antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), respectively. The primary antibodies or normal blocking serum (Dako Corporation, Inc., Carpinteria, CA, USA) was added and incubated overnight at 4°C. Biotinconjugated rabbit anti-rat IgG (Dako, Ely, UK), diluted to 1:400, was used as the secondary antibody and incubated during 30 min at 37˚C. An avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, USA) was consecutively added and incubated for 20 min, and the reaction was developed with DAB (Sigma Chemical Corporation). Omission of primary antibody served as a negative control. The slides were counterstained with anti-mouse α-smooth actin (Sigma Chemical Corporation), diluted to 1:600. Finally, cover sections with a glass coverslip (Merck Ltd, Lutterworth, Germany) were observed under an optical microscope. Immunohistochemistry staining intensity was evaluated in a blinded manner and scored on a percentage positive area for TNF-α (identified by brown staining) and the cells per area demonstrating nuclear localization for NF-κB were automatically detected on 10 different fields and averaged.

2.9. Data analysis

All grouped data were expressed as the means ± S.E. and one-way ANOVA was used for the comparisons. Subsequent analyses were performed using Newman-Keuls method. The developed tension-length relation was evaluated by linear regression analysis. The values of developed tension at each resting length were compared by one-way ANOVA. The resting length-tension curves were fit to a monoexponential relation, as follows: $y = \beta_0 \cdot e^{\beta 1 x} + \varepsilon_i$, where β_0, β_1, and ε_i are constants of the curve. These non-linear relations were compared between groups by the values of the constant stiffness, β_1.

Table 1. Influence of isoproterenol and exercise training on LV morphological and function.

Variables	Experimental groups				P value
	Con n = 10	Iso n = 13	Ex n = 8	Iso+Ex n = 8	
Body weight (g)	367 ± 13	359 ± 7	347 ± 21	349 ± 16	0.70
CSA (mm^2)	0.77 ± 0.04	1.30 ± 0.02*	0.67 ± 0.02	0.65 ± 0.02	<0.001
Echocardiography					
LVPWd (mm)	1.44 ± 0.10	1.92 ± 0.13*	1.46 ± 0.06	1.54 ± 0.09	<0.05
LVPWs (mm)	2.50 ± 0.12	3.34 ± 0.15*	2.46 ± 0.11	2.56 ± 0.07	<0.001
LVEDD (mm)	7.66 ± 0.21	7.11 ± 0.17	7.66 ± 0.34	7.97 ± 0.18	0.06
LVESD (mm)	4.52 ± 0.16	3.12 ± 0.20*	4.63 ± 0.32	5.03 ± 0.15	<0.001
FS (%)	41 ± 1	56 ± 2*	40 ± 2	37 ± 1	<0.001
Hemodynamics					
HR (beats/min)	401 ± 9	483 ± 20*	415 ± 10	386 ± 15	<0.01
LVSP (mmHg)	127 ± 2	116 ± 2*	124 ± 3	131 ± 3	<0.05
LVEDP (mmHg)	7.9 ± 0.5	15.2 ± 0.9*	6.8 ± 0.8	7.3 ± 0.9	<0.001
+dP/dt (mmHg/sec)	8023 ± 175	10090 ± 354*	8122 ± 163	8313 ± 129	<0.001
–dP/dt (mmHg/sec)	6342 ± 296	6946 ± 331	6569 ± 215	6516 ± 156	0.43
MAP (mmHg)	101 ± 3	84 ± 3*	103 ± 3	106 ± 3	<0.01

CSA, cross-section area of papillary muscles; LVPWd, diastolic LV posterior wall thickness; LVPWs, systolic LV posterior wall thickness; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; FS, LV fractional shortening; HR, heart rate; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; +dP/dt, maximum positive time derivative of developed pressure; –dP/dt, maximum negative derivative of developed pressure; MAP, mean arterial pressure. * Significant differences in ANOVA *vs.* other groups.

3. RESULTS

3.1. Exercise training inhibits isoproterenol-induced myocardial hypertrophy

The body weight was similar among the groups (Table 1). The administration of isoproterenol over an 8-day period resulted in a significant increase in cardiac and LV mass between Iso and the other groups (Figure 1). Exercise training did not affect the myocardial mass and, more important, completely blunted the hypertrophic response to β-adrenergic stimulation.

3.2 Exercise training effects on LV structure and function after β-adrenergic overload

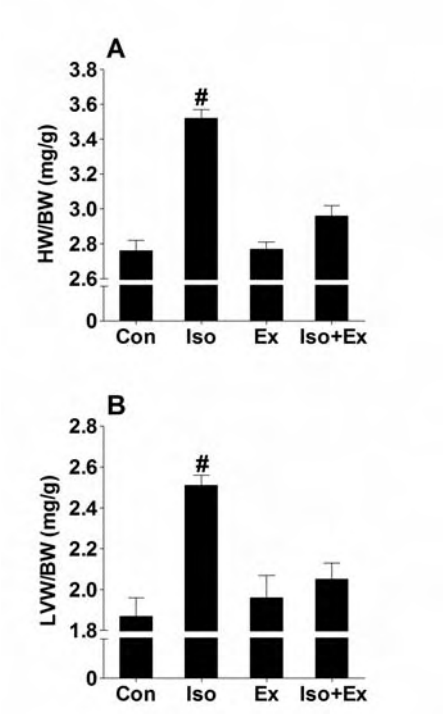

Figure 1. Effects of exercise training on the myocardial hypertrophy induced by isoproterenol. A: heart weight / body weight (HW/BW) for non-trained (Con, n = 12; Iso, n = 13) and exercise-trained rats (Ex, n = 8; Iso+Ex, n = 8) determined after 13 weeks of follow-up. B: left ventricular weight / body weight (LVW/BW) ratio for all groups. Significant differences in ANOVA, # $P<0.001$ *vs.* other groups.

On transthoracic echocardiography (Table 1), the isoproterenol induced changes similar to concentric hypertrophy: significant thickening of LVPWd and limitrophe significant reduction of LVEDD. Moreover, the β-adrenergic overload resulted in a remarkable increase in FS (+36%) in treated, non-trained rats compared to other groups. On LV hemodynamics measurements (Table 1), the heart rate and +dP/dt were significantly higher in the Iso group compared to the Con group. The Iso group also showed a reduction in LVSP and mean arterial pressure. In addition, there was marked left-ventricular diastolic dysfunction, as assessed by the exacerbated increase in LVEDP. Exercise training did not affect LV structure and function but, interestingly, prevented the alterations provoked by isoproterenol.

3.3. Exercise training enhanced the myocardial mechanics even in isoproterenol-treated animals.

There were striking differences in papillary muscle morphology between the non-trained group treated with isoproterenol and the other groups. Indeed, CSA was significantly greater (Table 1) in the Iso group compared with the Con group. Exercise training by itself did not alter the papillary muscle CSA, and notably it was effective in maintaining normal values of injected rats.

As shown in Figure 2, there were remarkable differences in myocardial performance between the four groups. We confirmed findings of previous studies [5,6] in which the sustained administration of isoproterenol resulted

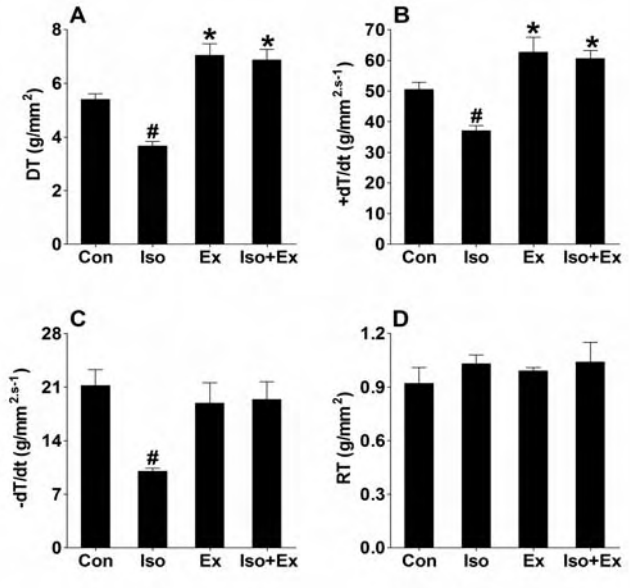

Figure 2. Exercise training increases the myocardial performance, even after β-adrenergic hyperactivity. Data obtained at muscle lengths corresponding to 100% of Lmax from non-trained (Con, n = 8; Iso, n = 13) and exercise-trained rats (Ex, n = 8; Iso+Ex, n = 6) as described in Materials and Methods. **A:** peak developed tension (DT). **B:** maximum positive time derivative of developed tension (+dT/dt). **C**, maximum negative time derivative of developed tension (–dT/dt). **D:** resting tension (RT). Significant differences in ANOVA, # P< 0.05 *vs.* other groups, * P<0.05 *vs.* Con group.

in muscle that developed less force than their respective control; the effect is depicted as a decrease in DT, as well as a smaller +dT/dt. Furthermore, –dT/dt was significantly diminished in β-adrenergically stimulated non-trained rats compared with non-trained rats that received only vehicle. The myocardial contractile performance was significantly enhanced after the exercise training program. Indeed, papillary muscles from the Ex group exhibited a significant increase in DT and +dT/dt. Moreover, exercise training more than just preserved the myocardial performance after β-adrenergic hyperactivity; the contractile properties were markedly improved in the Iso+Ex group as assessed by DT and +dT/dt. In addition, –dT/dt of exercise-trained rats was maintained, even when rats were treated with isoproterenol. The RT was not altered by isoproterenol or exercise training.

Additionally, as shown in Figure 3, the DT was plotted as a function of muscular

length. There was a linear relationship between DT and muscle length for all experiments, as evidenced by the coefficient of determination (r^2), which was typically > 0.97. The active length-tension relation from the Iso group was shifted downward with a reduced slope of linear regression in relation to the other groups. Notably, the slope of the linear projection of tension was preserved in exercise-trained rats after chronic β-adrenergic stimulation. This way, it is possible to define that for the same variation of muscle length, the recruitment of the Frank-Starling mechanism is preserved in trained myocardium after β-adrenergic hyperactivity. Furthermore, the length-tension curves from the exercise-trained groups (Ex and Iso+Ex) were shifted upward from that of the Con and Iso groups, indicating that, even though the Frank-Starling mechanism was unchanged, for the same stretch level, greater muscle force is generated in exercised rats (Figure 3). The resting length-tension curves were similar between the groups as evaluated by mean values of β_1, reflecting that isoproterenol as well as exercise training did not affect myocardial stiffness (Figure 3).

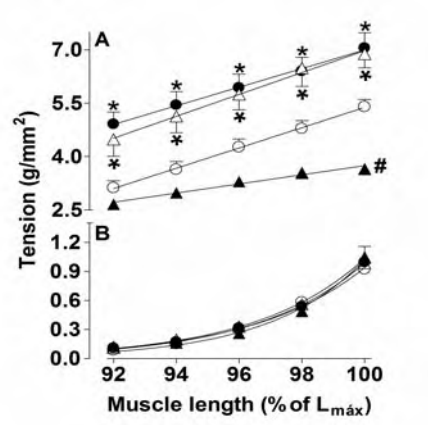

Figure 3. Left ventricular papillary muscle developed and resting length-tension curves obtained from Con (○), Iso (▲), Ex (●), and Iso+Ex (Δ) groups as described in Materials and Methods. **A:** straight lines were fit to the developed length-tension relations using linear regression analysis. The resulting means slopes corresponded to developed tension were compared between groups. ANOVA and *post hoc* Newman-Keuls test were used for multiple comparisons. # P<0.001 when slope was compared to others groups. * P<0.01 when the developed tension was compared to Con and Iso groups for each stretching. **B:** the resting tension-length curves for the four groups were fit to monoexponential non-linear relations. The resulting means β1 corresponded to resting tension were compared between groups.

3.4. Effects of exercise training on myocardial cytokines gene expression induced by isoproterenol

It is well known that adrenergic nervous system activation acts as a powerful stimulus for myocardial inflammatory cytokines synthesis [17]. Thus, β-adrenergic hyperactivity significantly increased TNF-α and IL-6 mRNA in the Iso group compared with the Con group (Figure 4). More important, exercise training did not affect local cytokines and inhibited the TNF-α and IL-6 increases induced by isoproterenol. Moreover, the

exercise training showed to increase the IL-10 mRNA expression in isoproterenol-affected myocardium. Remarkably, in the trained treated rats, the ratios of TNF-α/IL-10 and IL-6/IL-10 were maintained (Figure 4).

Consistent with other observations in that chronic β-adrenergic activation is thought to promote hypertrophy via local stimulation of TGF-β_1 [7], isoproterenol treatment increased the TGF-β_1 mRNA level ≈ 15.6-fold in Iso group as compared to Con group (Figure 4). As shown by quantitative RT-PCR analysis, the exercise training did not resulted in significant modification on expression of TGF-β_1 mRNA, yet the physical conditioning resulted in attenuation of TGF-β_1 mRNA increase in rats submitted to isoproterenol.

3.5. Effects of exercise training on myocardial cytokines protein expression induced by isoproterenol

The western blot results are shown in Figure 5. Consistent with their mRNA levels, TNF-α and IL-6 proteins were significantly raised in the Iso group and the exercise training was able to inhibit the TNF-α and IL-6 increase induced by isoproterenol. The level of IL-10 protein was significantly increased only in the

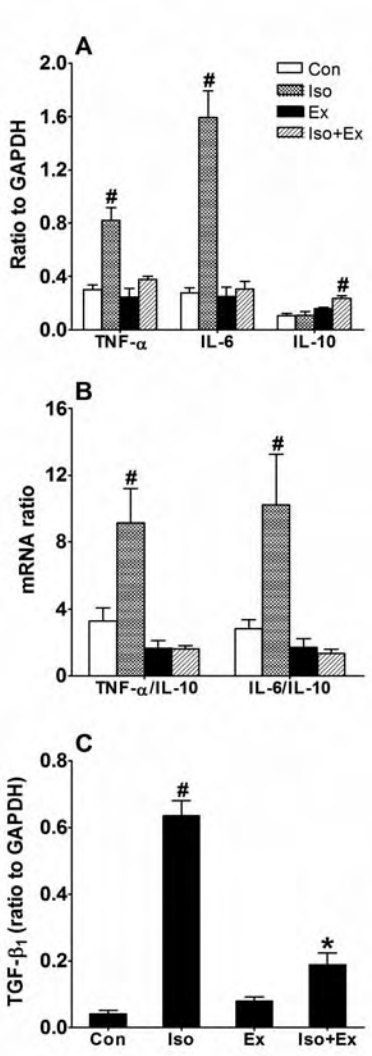

Figure 4. Gene expression by real-time RT-PCR in myocardium as described in Materials and Methods. **A:** gene expression of pro (TNF-α and IL-6) and anti (IL-10)-inflammatory cytokines. **B:** ratio of TNF-α/IL-10 and IL-6/IL-10. **C:** gene expression of TGF-β_1. All values were normalized for levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data are from five rats in each group. ANOVA and *post hoc* Newman-Keuls test were used for multiple comparisons. # $P<0.05$ *vs.* other groups. * $P<0.05$ *vs.* Con and Ex groups.

Ex group. Furthermore, as well as in the analyses of gene expression, the TNF-α/IL-10 and IL-6/IL-10 ratios in the Ex and Iso+Ex groups were similar to that of

the Con group, but were significantly higher in the Iso group.

3.6. Localization of pro-inflammatory (TNF-α) cytokine in myocardium

The imunostaining revealed intense immunoreactivity for TNF-α in isoproterenol myocardium in a diffuse manner (Figure 6). Positive imunostaining was noted in areas of inflammatory cell infiltration and in the myocardial tissue distinct from these areas. Importantly, positive immunoreactivity for TNF-α was rarely detected in Con and Ex groups as well as in the Iso+Ex group. The cardioprotector effect was confirmed by the TNF-α immunoreactivity scores.

3.7. Effects of isoproterenol and exercise training on nuclear NF-κB expression

Because activation of NF-κB may participate in the development of myocardial hypertrophy-induced by isoproterenol [18-20], nuclear NF-κB expression was evaluated in the rats treated with isoproterenol. As shown in Figure 6, β-adrenergic hyperactivity significantly increased nuclear NF-κB expression by ≈ 4.1-fold in the Iso group as compared with the Con group. Exercise training did not increase NF-κB expression. Nonetheless, exercise

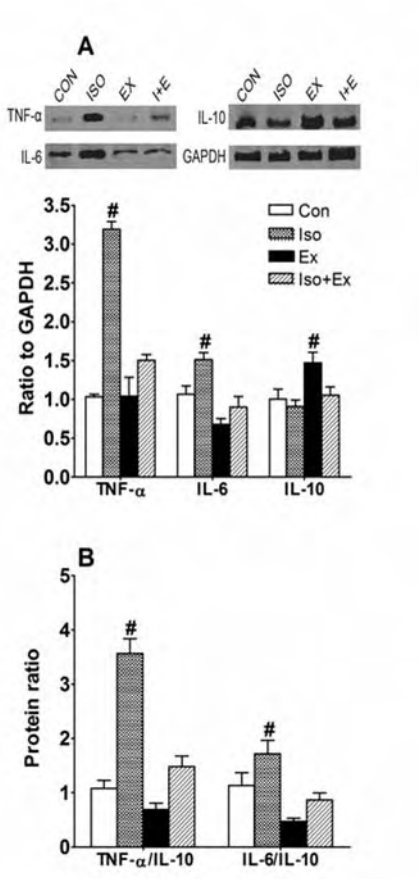

Figure 5. The protein expression by western blot in myocardium as described in Materials and Methods. **A:** protein expression of TNF-α, IL-6 and IL-10. The upper panel is a representative western blot. **B:** ratio of TNF-α /IL-10 and IL-6/IL-10. All values were normalized for levels of GAPDH. Data are from five rats in each group. Data are from five rats in each group. ANOVA and *post hoc* Newman-Keuls test were used for multiple comparisons. # P<0.05 *vs.* other groups

training preceding isoproterenol administration partially blunted the expression of NF-κB increase, although not significantly.

4. DISCUSSION

Recently, we demonstrated the cardioprotection effects of exercise training against LV lesions induced by

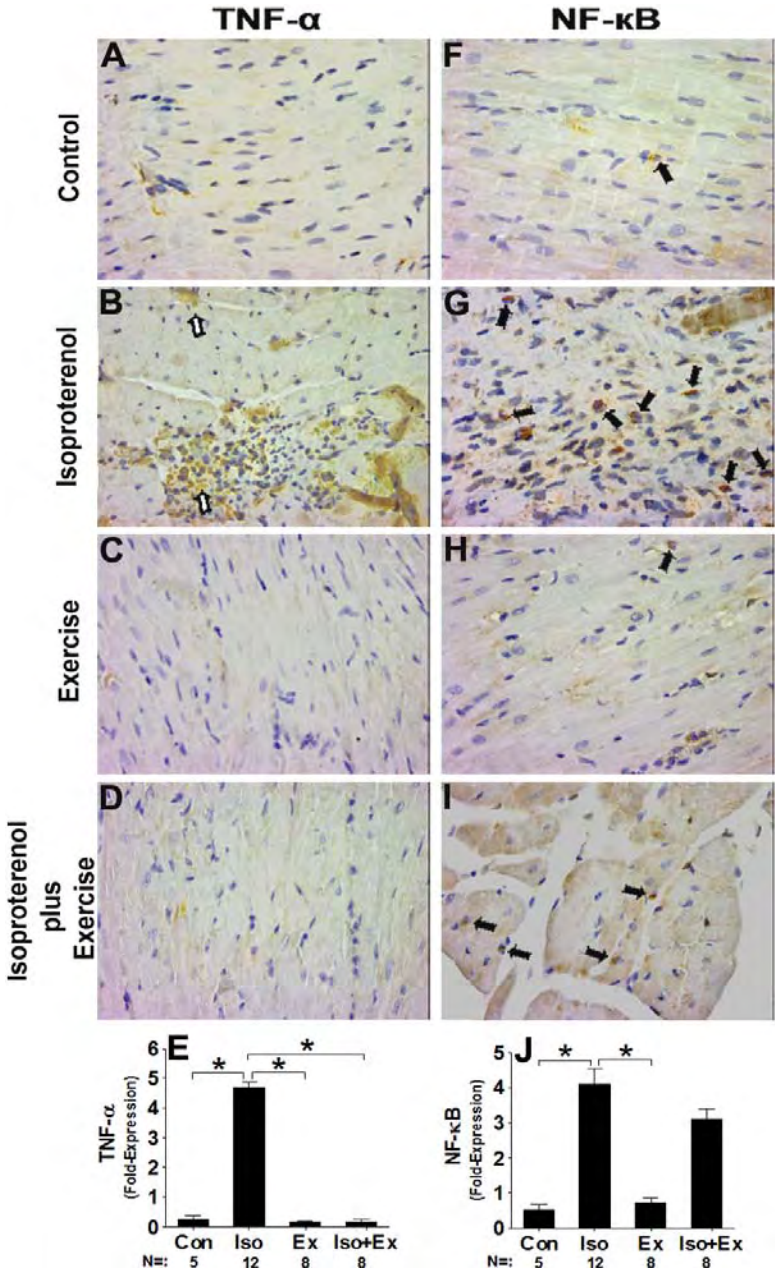

Figure 6. A representative photomicrograph showing localization of TNF-α and NF-κB in myocardium. Brown stain indicates positive immunoreactivity. In left column, intense immunoreactivity was detected in sedentary isoproterenol-treated animals (**B**). To notice that the TNF-α was readily positive and localized to areas of inflammatory cell infiltration and myocardial tissue no injured (empty arrows). However, in trained animals the exercise training was able to inhibit the intense pro-inflammatory staining after isoproterenol overload. Thereby, positive immunoreactivity to TNF-α was rarely detected in myocardium of Con (**A**), Ex (**C**) and Iso+Ex (**D**) groups. In right column, the full arrows indicate positive nuclear immunoreactivity for NF-κB in Con (**F**), Iso (**G**), Ex (**H**) and Iso+Ex (**I**) groups. **E** and **J**, statistical analysis for TNF-α and NF-κB, respectively. Numbers of rats used in each group and fold-expression relative to the Con group are shown. * $P<0.05$ in ANOVA. Magnification x400.

prolonged stimulation with isoproterenol [8], yet the myocardial function and possible mechanisms for this occurrence were not established. The present study provides novel information in this regard. We found that the myocardial dysfunction induced by β-adrenergic hyperactivity was prevented by exercise training. This maintenance of myocardial function was accompanied by inhibition of myocardial gene and protein overexpression of TNF-α and IL-6 as well as an improvement in the anti-inflammatory IL-10 expression. Furthermore, the exercise training attenuated the increase of TGF-β_1 mRNA and nuclear NF-κB expression, a well-known growth factor and a transcriptional regulator involved in the β-adrenergic signalling, respectively [7,18-20].

As previously stated, the sedentary rats submitted to isoproterenol showed a high increase in myocardial mass and it is usually characterized by a combination of muscle fiber enlargement, replication of non-myocytes cells and myocardial edema [7-8]. Moreover, on echocardiography, the aforementioned rats showed chamber dimensions indicating that the isoproterenol-induced LV hypertrophy was of the concentric type, which corroborates previous findings in our lab [8,21].

Sustained β-adrenergic activation is thought [7] to promote myocardial hypertrophy via local stimulation of myocardial growth factors, up-regulation of proto-oncogenes, increase in oxidative stress, activation of mitogen-activated protein kinases, and phosphatidyinositol 3-kinase signalling. In this study, we identified possible signalling triggers involved in the myocardial remodeling induced by β-adrenergic hyperactivity. Our results confirm that isoproterenol is a powerful stimulant to local myocardial expression of TNF-α and IL-6 [17]. Furthermore, TNF-α immunoreactivity was not confined to regions of inflammatory cell infiltration, but was present even in preserved tissues, suggesting direct expression of cytokines by non-inflammatory cells [17]. The interplay between β-adrenergic hyperactivity and overexpression of TNF-α and IL-6 may have implications with respect to adverse cardiac remodeling. Indeed, pro-inflammatory cytokines were shown to cause myocyte apoptosis, extracellular matrix alterations, contractile depression and

concentric hypertrophy that evolves to dilated cardiomyopathy over time [22-24].

Cardiac hypertrophy is associated with an increased expression of TGF-β_1 mRNA [25]. The *in vivo* researches with transgenic animals have been confirmed that TGF-β_1 is implicated in the cardiac hypertrophy. Rosenkranz et al. [26] showed that mice overexpressing TGF-β_1 revealed significant cardiac hypertrophy, accompanied by an increased expression of hypertrophy-associated genes. Several lines of evidence suggest a link between β-adrenergic system and the TGF-β_1 signalling [27-29]. In accordance with previous studies [28,29], we also showed that TGF-β_1 mRNA myocardial levels were raised after isoproterenol infusion. Although this findings lead to important participation of the TGF-β_1 in the cardiac hypertrophy induced by β-adrenergic hyperactivity, we cannot exclude the possibility that the cardiac alterations may at least in part have occurred due to other signaling pathways. It should be cited that a positive correlation has been found between elevated myocardial TGF-β_1 mRNA expression and increased heart weight-to-body weight ratio [29].

The β-adrenergic hyperactivity increased the nuclear myocardial expression of NF-κB, a ubiquitous transcription factor, which is known for its role in cell growth, inflammation, apoptosis, and embryonal development [18]. The NF-κB role in inducing hypertrophic phenotypic during β-adrenergic stimulation has been demonstrated by Freund et al. [18], when generated mice with cardiomyocyte-restricted expression of the NF-κB super-repressor IkappaBalphaDeltaN displayed an attenuated hypertrophic response to 7 days of isoproterenol treatment. Similarly, Chandrasekar et al. [19] and Takemoto et al. [20] showed that isoproterenol resulted in a significant increase in NF-κB-dependent DNA binding activity in the myocardium of mice and rats, respectively.

Of clinical relevance, we showed that exercise training inhibited the LV remodeling in response to a pathologic stimulus. The mechanism (s) responsible for this exercise-induced cardioprotection against β-adrenergic agonists has yet to be fully defined, yet this study shows that the myocardial TNF-α and IL-6 increase evoked by isoproterenol was completely abolished by exercise training and this might have

contributed to the cardioprotection effects of exercise training. By the way, the exercise training maintained TNF-α/IL-10 and IL-6/IL-10 ratios in normal levels. Consequently, exercise training may affect the LV remodeling after β-adrenergic overload by modulating the balance between pro-inflammatory *vs*. anti-inflammatory cytokines. Furthermore, the TGF-β_1 and NF-κB signaling provoked by β-adrenergic hyperactivity was reduced in the exercised rats and this is in agreement with the current idea that exercise training may be an important modulator of growth factors and/or transcriptional regulators in pathologic cardiac hypertrophy [30].

The β-adrenergic hyperactivity for the non-trained rats was associated with concentric LV hypertrophy with increase of FS and +dP/dt, suggesting an illusory improvement of the LV contractile state. Nevertheless, we evaluated the myocardial contractile capacity on the papillary muscle preparation and, in this approach; our results are consistent with a depressed contractile state after adrenergic hyperactivity. Moreover, the isoproterenol provoked a shift downward of length/active tension curves, suggesting that Frank-Starling relationship is worsened [31]. Thus, taking into account that concentric hypertrophy facilitates pressure generation (Laplace's law) by increasing mass/volume ratio [21], it is conceivable that FS and +dP/dt should be higher in hypertrophied LV in spite of force generation impairment. In addition, there was diastolic dysfunction as assessed by an increase of LVEDP and –dT/dt reduction in non-trained rats treated with isoproterenol. Perhaps the most likely candidate to be appointed as responsible for mediating isoproterenol-induced diastolic dysfunction is an imbalance between muscular tissue and collagenous compartments [8,31-33]. Moreover, the increase of ventricular diastolic stiffness is a common consequence of concentric hypertrophy [34] and, additionally, there are data demonstrating that β-adrenergic hyperactivity induces deleterious alterations in the expression and/or phosphorylation of regulatory cardiac proteins, e.g., phospholamban and sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase, as well as a reduced adenosine 3',5'-cyclic monophosphate accumulation [6].

Exercise training has been shown to be

effective in improving the functional characteristics of hearts from hypertensive [35], myocardial-infarcted [16, 36] and aged rats [37]. However, this is the first report documenting that exercise training can prevent functional abnormalities from chronically β-adrenergically stimulated rats. It is noteworthy that the β-adrenergic hyperactivity did not inhibit the beneficial effect of exercise training on myocardial contractile capacity.

Clinical Implications

The present study indicates that the exercise training started before β-adrenergic hyperactivity insult is beneficial, resulting in LV hypertrophy prevention and improved myocardial performance, without adverse effects on chamber remodeling. Our data showed that exercise training is very effective in promoting heart protection against β-adrenergic hyperactivity, recommended as a part of the gold standard treatment for attenuation of ventricular remodeling and heart failure [38]. The exercise training can offer protection by a positive balance between anti-inflammatory *versus* pro-inflammatory cytokines as well as by modulation of TGF-β_1 signaling in pathologic cardiac hypertrophy. Nevertheless, future studies should be aimed at investigating whether combined β-adrenoreceptor blockade and exercise training yield added benefit. For clinical purposes, assuming that the isoproterenol dose used in our study is excessive in terms of usual physiologic β-adrenergic stimulation, exercise training can be considered as very effective in cardioprotection against β-adrenergic hyperactivity.

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5. REFERENCES

1. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med

1990;322:1561-66.

2. Lips DJ, deWindt LJ, van Kraaij DJ, Doevendans PA. Molecular determinants of myocardial hypertrophy and failure: alternative pathways for beneficial and maladaptive hypertrophy. Eur Heart J 2003;24:883-96.

3. Zou Y, Komuro I, Yamazaki T, Kudoh S, Uozumi H, Kadowaki T, et al. Both G_s and G_i proteins are critically involved in isoproterenol-induced cardiomyocyte hypertrophy. J Biol Chem 1999;274:9760-70.

4. Brum PC, Kosek J, Patterson A, Bernstein D, Kobilka B. Abnormal cardiac function associated with sympathetic nervous system hyperactivity in mice. Am J Physiol Heart Circ Physiol 2002;283:H1838-45.

5. Vassallo DV, Vasquez EC, Cabral AM. Contractile performance of papillary muscles of renovascular hypertensive and isoproterenol-pretreated rats. Pharmacol Res Commun 1988;20:61-72.

6. Stein B, Bartel S, Kirchhefer U, Kokott S, Krause EG, Neumann J, et al. Relation between contractile function and regulatory cardiac proteins in hypertrophied hearts. Am J Physiol Heart Circ Physiol 1996;270:H2021-28.

7. Osadchii OE. Cardiac hypertrophy induced by sustained beta-adrenoreceptor activation: pathophysiological aspects. Heart Fail Rev 2007;12:66-86.

8. Serra AJ, Higuchi ML, Ihara SS, Antônio EL, Santos MH, Bombig MT, et al. Exercise training prevents beta-adrenergic hyperactivity-induced myocardial hypertrophy and lesions. Eur J Heart Fail 2008;10:534-39.

9. Brouri F, Hanoun N, Mediani O, Saurini F, Hamon M, Vanhoutte PM, et al. Blockade of beta 1- and desensitization of beta 2-adrenoreceptors reduce isoprenaline-induced cardiac fibrosis. Eur J Pharmacol 2004;485:227-34.

10. Gallego M, Espiña L, Vegas L, Echevarria E, Iriarte MM, Casis O. Spironolactone and captopril attenuates isoproterenol-induced cardiac remodelling in rats. Pharmacol Res 2001;44:311-15.

11. Okuda N, Hayashi T, Mori T, Inamoto S, Okabe M, Mieno S, et al. Nifedipine enhances the cardioprotective effect of an angiotensin-II receptor blocker in an experimental animal model of heart failure. Hypertens Res 2005;28:431-38.

12. Ishizawa M, Mizushige K, Noma T,

Namba T, Guo P, Murakami K, et al. An antioxidant treatment potentially protects myocardial energy metabolism by regulating uncoupling protein 2 expression in a chronic beta-adrenergic stimulation rat model. Life Sci 2006;78:2974-82.

13. Riggs CE Jr, Landiss CW, Jessup GT, Bonner HW. Effects of exercise on the severity of isoproterenol-induced myocardial infarction. Med Sci Sports 1977;9:83-7.

14. Brodowicz GR, Lamb DR. Exercise training, indomethacin, and isoproterenol induced myocardial necrosis in the rat. Basic Res Cardiol 1991;86:40-8.

15. Darrah MI, Engen RL. Beneficial effects of exercise on l-isoproterenol induced myocardial infarction in male rats. Med Sci Sports Exerc 1982;14:76-80.

16. Portes LA, Saraiva RM, Santos AL, Tucci PJF. Swimming training attenuates remodeling, contractile dysfunction and congestive heart failure in rats with moderate and large myocardial infarctions. Clin Exp Pharmacol Physiol 2009;36:394-9.

17. Murray DR, Prabhu SD, Chandrasekar B. Chronic beta-adrenergic stimulation induces myocardial proinflammatory cytokine expression. Circulation 2000;101:2338-41.

18. Freund C, Schmidt-Ullrich R, Baurand A, Dunger S, Schneider W, Loser P, et al. Requirement of nuclear factor-kappaB in angiotensin II- and isoproterenol-induced cardiac hypertrophy in vivo. Circulation 2005;111:2319-25.

19. Chandrasekar B, Marelli-Berg FM, Tone M, Bysani S, Prabhu SD, Murray DR. Beta-adrenergic stimulation induces interleukin-18 expression via beta2-AR, PI3K, Akt, IKK, and NF-kappaB. Biochem Biophys Res Commun 2004;319:304-11.

20. Takemoto Y, Yoshiyama M, Takeuchi K, Omura T, Komatsu R, Izumi Y, et al. Increased JNK, AP-1 and NF-κappaB DNA binding activities in isoproterenol-induced cardiac remodeling. J Mol Cell Cardiol 1999;31:2017-30.

21. Murad N, Tucci PJ. Isoproterenol-induced hypertrophy may result in distinct left ventricular changes. Clin Exp Pharmacol Physiol 2000;27:352-7.

22. Janczewski AM, Kadokami T, Lemster B, Frye CS, MacTiernan CF, Feldman AM. Morphological and functional changes in cardiac myocytes

isolated from mice overexpressing TNF-alpha. Am J Physiol Heart Circ Physiol 2003;284:H960-9.

23. Sivasubramanian N, Coker ML, Kurrelmeyer KM, MacLellan WR, DeMayo FJ, Spinale FG, et al. Left ventricular remodeling in transgenic mice with cardiac restricted overexpression of tumor necrosis factor. Circulation 2001;104:826-31.

24. van Empel VP, De Windt LJ. Myocyte hypertrophy and apoptosis: a balancing act. Cardiovasc Res 2004;63:487-99.

25. Li RK, Mickle DA, Weisel RD, Merante F, Luss H, Rao V, et al. Overexpression of transforming growth factor-beta1 and insulin-like growth factor-I in patients with idiopathic hypertrophic cardiomyopathy. Circulation 1997;96:874-81.

26. Rosenkranz S, Flesch M, Amann K, Haeuseler C, Kilter H, Seeland U, et al. Alterations of beta-adrenergic signaling and cardiac hypertrophy in transgenic mice overexpressing TGF- beta1. Am J Physiol Heart Circ Physiol 2002;283:H1253-62.

27. Masson S, Arosio B, Luvarà G, Gagliano N, Fiordaliso F, Santambrogio D, et al. Remodelling of cardiac extracellular matrix during beta-adrenergic stimulation: upregulation of SPARC in the myocardium of adult rats. J Mol Cell Cardiol 1998;30:1505-14.

28. Omura T, Kim S, Takeuchi K, Iwao H, Takeda T. Transforming growth factor beta 1 and extracellular matrix gene expression in isoprenaline induced cardiac hypertrophy: effects of inhibition of the renin-angiotensin system. Cardiovasc Res 1994;28:1835-42.

29. Boluyt MO, Long X, Eschenhagen T, Mende U, Schmitz W, Crow MT, et al. Isoproterenol infusion induces alterations in expression of hypertrophy-associated genes in rat heart. Am J Physiol Heart Circ Physiol 1995;269:H638-47.

30. Konhilas JP, Watson PA, Maass A, Boucek DM, Horn T, Stauffer BL, et al. Exercise can prevent and reverse the severity of hypertrophic cardiomyopathy. Circ Res 2006;98:540-8.

31. Matsubara LS, Matsubara BB, Okoshi MP, Cicogna AC, Janicki JS. Alterations in myocardial collagen content affect rat papillary muscle function. Am J Physiol Heart Circ Physiol 2000;279:H1534-9.

32. Hanft LM, Korte FS, MacDonald KS. Cardiac function and modulation of sarcomeric function by length.

Cardiovasc Res 2008;77:627-36.

33. Jalil JE, Doering CW, Janicki JS, Pick R, Shroff SG, Weber KT. Fibrillar collagen and myocardial stiffness in the intact hypertrophied rat left ventricle. Circ Res 1989;64:1041-50.

34. Vangheluwe P, Tjwa M, Bergh AVD, Louch WE, Beullens M, Dode L, et al. A Serca 2 pump with an increased Ca^{2+} affinity can lead to severe cardiac hypertrophy, stress intolerance and reduced life span. J Mol Cell Cardiol 2006;41:308-17.

35. Morris RT, Fine DM, Lees SJ, Booth FW, Link CD, Ferrario CM, et al. Exercise training prevents development of cardiac contractile dysfunction in hypertensive TG (mREN-2)27 rats. J Am Soc Hypertens 2007;1:393-9.

36. Leosco D, Rengo G, Laccarino G, Golino L, Marchese M, Fortunato F, et al. Exercise promotes angiogenesis and improves beta-adrenergic receptor signalling in the post-ischaemic failing rat heart. Cardiovasc Res 2008;78:385-94.

37. Barmeyer A, Müllerleile K, Mortensen K, Meinertz T. Diastolic dysfunction in exercise and its role for exercise capacity. Heart Fail Rev 2009;14:125-34.

38. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, et al. ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure).

DISCUSSÃO

É bem conhecido que o aumento da atividade nervosa simpática está freqüentemente associado com adversidades ao coração. Destaque-se a HM patológica (Zierhut & Zimmer, 1989; Fleming et al, 1992; Zzou et al, 1999; Greenwood et al, 2001; schlaich et al, 2003; strand et al, 2006), que constitui um fator de risco poderoso para o desenvolvimento de insuficiência cardíaca (Kannel et al, 1987b; Levy et al 1990; Lips et al, 2003). Adicionalmente, na insuficiência cardíaca há ativação crônica do sistema simpático, que resulta em mudanças deletérias no coração, causando progressivo remodelamento e disfunção miocárdica com consequente progressão do fenótipo da insuficiência cardíaca (Kaye et al, 1995; Floras, 2003). Estes dados conferiram aos antagonistas beta-adrenérgicos um papel importante no panorama atual da terapêutica da HM e insuficiência cardíaca.

Quando o enfoque é analisar os mecanismos fisiopatológicos envolvidos na agressão simpática cardíaca, modelos experimentais de sobrecarga adrenérgica são de grande valia. Além disso, em condições experimentais, vieses de confusão como a idade, intensidade e duração do estimulo adrenérgico e a associação com outras doenças ou medicamentos podem ser mais bem controlados.

Em nosso estudo, optamos por um modelo experimental de sobrecarga β-adrenérgica prolongada com ISO. Tanto a dose como o período de administração preconizados já foram mostrados induzir um fenótipo de remodelamento miocárdico patológico similar ao identificável em humanos (Milei et al., 1978; Tang & Taylor, 1984a; Tang & Taylor, 1984b; Jalil et al, 1989). Assim, nós confirmamos (*artigo 1:* tabela 1) resultados prévios de nosso laboratório, obtidos com o mesmo protocolo de

administração de ISO, que mostrou promover expressivo aumento da massa miocárdica normalizada pelo peso corpóreo (Murad & Tucci, 2000). Considerando que na HM induzida pelo ISO há focos de necrose miocárdica, a veracidade da massa miocárdica em sinalizar o crescimento dos cardiomiócitos fica prejudicada. Consequentemente, os valores de volume nuclear e diâmetro transverso da fibra cardíaca também foram analisados e confirmaram o fenômeno de remodelamento hipertrófico (*artigo 1:* tabela 1). Tendo em mente tais dados, somados às avaliações histomorfométricas da espessura de parede e diâmetro transverso da cavidade ventricular esquerda, é possível inferir que a administração do ISO evolui com HM do tipo concêntrica.

A literatura dispõe de informações que apontam para ampla gama de mecanismos subcelulares possivelmente envolvidos com a acentuação da síntese protéica quando do uso de ISO. Admite-se que o agonismo dos receptores β-adrenérgicos na membrana plasmática conduza à ativação da proteína quinase A via adenilciclase, o que, constitui uma via de sinalização para ativação de protooncogenes diretamente envolvidos com o processo de transcrição (Morgan & Baker, 1991; Brand et al, 1993, Sugden & Bogoyevitch, 1995). Além desta via de sinalização, a HM induzida pelo ISO pode requerer a ativação da proteína quinase C, via ação da angiotensina II (Grimm et al, 1998; Grimm et al, 1999; Gallego et al, 2001). outros fatores de crescimento, incluindo o TGF-β1 (Boluyt et al, 1995; Osadchii, 2007a), o fator 1 de crescimento ligado a insulina (oudit et al, 2003) e as proteínas quinases ativadoras de mitogenia (Zou et al, 2001;Zhang et al, 2005), ao que parece, também sinalizam os efeitos hipertróficos do ISO.

Também derivam de nossos resultados a confirmação de achados prévios da literatura (Rona et al, 1959; Rona et al, 1963; Alderman & Harrison, 1971; Haft, 1974;

Taylor et al, 1977; Milei et al, 1978; Benjamin et al, 1989; Jalil et al, 1989; Ljubuncić et al, 1991; Ljubuncić et al, 1992a; Ljubuncić et al, 1992b; Ljubuncić et al, 1992c; Tang & Taylor, 1996; Gallego et al, 2001; Dudnakova et al, 2002; Ennis et al, 2003; Liu et al, 2009), em que foi patente nos animais sedentários tratados com ISO a existência de acentuada necrose miocárdica, com presença de infiltrado inflamatório, predominantemente na região subendocárdica (*artigo 1:* figura 1B). Em uma análise mais apurada, com o uso de microscópio eletrônico (*artigo 1:* figura 2B), foi possível verificar marcante desarranjo e lise dos miofilamentos em associação com ruptura da membrana plasmática. Em que pese a unanimidade das informações precedentes em torno da importância da necrose miocárdica, sua participação no processo de perda de cardiomiócitos induzida pelo ISO não é única. A possibilidade de a perda de miócitos decorrer de um processo de morte celular programada (apoptose) também é proeminente. O nosso encontro de aumento do número de núcleos em apoptose nos ratos sedentários (*artigo 1:* figura 3) justifica os relatos da literatura que são concordes em apontar a importância da apoptose na patogenesia miocárdica causada pelo ISO (Shizukuda et al, 1998; Tomita et al, 2003; Goldspink et al, 2004; Gálvez et al, 2005; Fan et al, 2006). Desta forma, em nossos experimentos, parece lícito considerar que a necrose e a apoptose coexistam para reduzir o número de cardiomiócitos no miocárdio submetido à sobrecarga β-adrenérgica e, por conseguinte, resultar em reduzida fração de área miofibrilar (*artigo1:* tabela 1).

A patogenia da injúria miocárdica induzida pelo ISO parece envolver múltiplos mecanismos, incluindo: aumento na geração de espécies reativas de oxigênio (Ishizawa et al, 2006; Zhou et al, 2006), sobrecarga intracelular de cálcio via adenosina monofosfato

cíclica (Bloom & Cancilla, 1969; Bloom & Davis, 1972; Mann, 1998), ativação do sistema renina-angiotensina-aldosterona (Grimm et al, 1998), ação local exacerbada de citocinas pró-inflamatórias (Murray et al, 2000; Chandrasekar et al, 2004), ativação de proteínas quinases ativadoras de mitogenia (Zou et al, 2001; Li et al, 2004), maior requerimento da via de sinalização da calcineurina (Saito et al, 2000) e estresse por isquemia/reperfusão (Osadchii, 2007a). Na luz de um possível fator etiológico para injúria tecidual evocada pelo ISO, é possível supor que a redução na fração de área capilar (*artigo 1:* tabela 1) nos animais sedentários possa resultar em menor quantidade de oxigênio viável ao miocárdio, assim, contribuindo para a perpetuação de necrose e apoptose dos cardiomiócitos.

O nosso encontro de aumento no conteúdo miocárdico de colágeno nos ratos sedentários (*artigo 1:* figura 1E) está em acordo com diversos trabalhos que abordaram a questão (Stanton & Bowman, 1967; Alderman & Harrison, 1971; Taylor et al, 1977; Benjamin et al, 1989; Jalil et al, 1989; Tang & Taylor, 1996; Gallego et al, 2001; Ennis et al, 2003). Apesar das informações microscópicas serem indicativas de um processo de fibrose miocárdica reparativa (Benjamin et al, 1989; Grimm et al, 1998), a importância da fibrose reativa concomitante, como decorrência da ação do sistema renina-angiotensina-aldosterona não pode ser descartada (Grimm et al, 1998; Grimm et al, 1999; Gallego et al, 2001).

A elucidação dos possíveis fatores etiológicos da injúria miocárdica desencadeada pelo ISO concebeu a realização de muitos estudos com objetivo de analisar qual (is) terapêutica farmacológica poderia ser efetiva em combater os efeitos cardiotóxicos decorrentes da sobrecarga β-adrenérgica. Antagonistas dos canais de cálcio (Yeager &

Whitehurst, 1982; Ljubuncić et al, 1992c; Okuda et al, 2005), do sistema renina-angiotensina-aldosterona (Ljubuncić et al, 1991; Nagano et al, 1992; Grimm et al, 1998; Gallego et al, 2001; Okuda et al, 2005), de receptores β-adrenérgicos (Ljubuncić et al, 1992a; Ljubuncić et al, 1992b; Woodiwiss et al, 2001; Brouri et al, 2004) e antioxidantes (Ishizawa et al, 2006; Liu et al, 2009), foram testados com sucesso na prevenção e/ou atenuação da injúria e disfunção miocárdica.

Experimentos iniciais despertaram novas perspectivas acerca da importância do EXF no combate aos eventos adversos ligados à sobrecarga β-adrenérgica. O primeiro trabalho, por nós localizado, que avaliou a influência do EXF sobre cardiotoxidade induzida pelo ISO, foi o de Tajuddin et al (1975). Os autores verificaram que ratos treinados em natação por período de oito semanas apresentaram menor concentração sérica de DHL e CQ após dois dias consecutivos de tratamento com 85 mg/kg/dia de ISO, achados estes, sugestivos de menor dano tecidual. Posteriormente, Crandall et al (1981), também utilizando a atividade plasmática da DHL como marcador de necrose miocárdica, verificaram que dez semanas de EXF em esteira atenuou significativamente a elevação da DHL de ratos que receberam 70 mg/kg/dia de ISO por dois dias consecutivos. Somado a isto, os autores constataram que o ISO não causou a morte de qualquer animal exercitado, enquanto que, no grupo que permaneceu sedentário, a taxa de mortalidade foi de 50%. Em acordo com os benefícios já mencionados do EXF, Darrah & Engen (1982) também noticiaram que não houve qualquer caso de morte em ratos treinados após a injeção de única dose de 70 mg/kg de ISO. Em contraposição, nós identificamos somente um estudo que não encontrou benefícios do EXF na injúria miocárdica evocada pelo ISO. Riggs et al (1977), administraram injeção única de 250

mg/kg de ISO em ratos corredores e não encontraram qualquer modificação nos níveis de atividade sérica da CQ, contudo, no mesmo estudo, os autores descreveram que a mortalidade foi significativamente reduzida nos animais treinados. Em estudo recente, Frederico et al (2009) documentaram que doze semanas de EXF prévio foram capazes de reduzir o estresse oxidativo, a CQ cardíaca e a mortalidade de ratos submetidos a dois dias de tratamento com 80 mg/kg/dia de ISO.

De extrema relevância são os nossos dados de proteção da hipertrofia e lesão miocárdicas nos animais previamente treinados e que receberam o ISO (*artigo 1*). Ao contrário dos estudos anteriores, que demonstraram efeito cardioprotetor parcial do EXF, nós observamos pleno sucesso do exercício em prevenir totalmente a injúria cardíaca secundária à sobrecarga β-adrenérgica. Os mecanismos que conduziram ao efeito cardioprotetor do EXF ainda precisam ser mais bem investigados, contudo, duas observações derivadas de nossos resultados podem ser satisfatoriamente consideradas: 1) nos animais exercitados não houve redução da fração de área capilar, o que pode ter contribuído para manutenção do aporte de oxigênio aos cardiomiócitos durante a administração do ISO e, consequentemente, participado da prevenção da isquemia tecidual; 2) considerando que a injúria celular associada à estimulação β-adrenérgica é dose-depedente (Teerlink et al, 1994), é presumível que os estudos anteriores não tenham identificado completa cardioproteção nos animais treinados devido à utilização de doses exorbitantes do ISO.

As informações precedentes fundamentam o posicionamento de que, apesar da dose de ISO usada em nosso estudo ser também excessiva em termos de estimulação β-adrenérgica usualmente fisiológica, o EXF pode ser considerado como uma intervenção

não farmacológica efetiva para prevenção das adversidades cardíacas ligadas à hiperatividade adrenérgica. Neste sentido, o efeito cardioprotetor do EXF foi observado em amplo aspecto e incluiu a prevenção de múltiplos fatores, dentre eles: HM, necrose, apoptose, inflamação, edema, fibrose subendocárdica, aumento do conteúdo de colágeno e redução das frações de área miofibrilar e capilar.

Depois da divulgação dos dados iniciais publicados no *European Journal of Heart Failure*, nosso interesse foi direcionado para avaliar os possíveis mecanismos subcelulares mediadores dos efeitos cardioprotetores do EXF frente à atividade β-adrenérgica sustentada. Além disso, nós analisamos o papel do EXF no controle da disfunção miocárdica desencadeada pelo ISO.

Como havíamos mostrado no primeiro estudo, nos animais sedentários houve aumento expressivo da massa miocárdica após oito dias de tratamento com ISO (*artigo 2:* figura 1). Estes achados foram acompanhados por significante aumento da espessura de parede do VE e redução da dimensão da cavidade ventricular esquerda (*artigo 2:* tabela 1). Novamente, este conjunto de medidas condicionou que, em nosso modelo de hiperatividade adrenérgica, o ISO promoveu hipertrofia ventricular concêntrica.

Em recente revisão da literatura (Osadchii, 2007a) ficou patente o envolvimento de múltiplas vias de sinalização responsáveis pela HM decorrente da hiperatividade β-adrenérgica sustentada. A estimulação local de fatores de crescimento, ativação de proto-oncogenes, aumento nas espécies reativas de oxigênio, ativação de proteínas quinases ativadores de mitogenia e sinalização via fosfatídil-inositol 3 quinase assumem grande relevância. Neste sentido, nós avançamos nossas análises com o intuito de identificar possíveis mediadores de sinalização envolvidos no remodelamento miocárdico induzido

pelo ISO.

Em 2000, Murray et al divulgaram que a estimulação β-adrenérgica com ISO durante sete dias foi capaz de promover aumento na expressão gênica e protéica das citocinas pró-inflamatórias, incluindo: fator de necrose tumoral alfa (TNF-α), interleucina 6 (IL-6) e interleucina 1 beta no miocárdio. Acresçam-se informações provenientes de nossos experimentos indicando que, na vigência do ISO, também houve aumento na expressão gênica (*artigo 2:* Figuras 4A) e protéica (*artigo 2:* figura 5A) do TNF-α e IL-6 no miocárdio dos animais sedentários, enquanto que, a expressão da interleucina 10 (IL-10) anti-inflamatória permaneceu aparentemente inalterada. Não obstante, intensa imunoreatividade positiva para o TNF-α foi observada em áreas de infiltração inflamatória e regiões preservadas (*artigo 2:* figura 6B) nos animais sedentários. Estas observações indicam produção direta das citocinas pró-inflamatórias pelos cardiomiócitos ou pelas células cardíacas não miocitárias (Murray et al, 2000).

Independentemente do proceder de sua ação ou produção, o fundamental é que ambos TNF-α e IL-6 resultam em remodelamento adverso do miocárdio. Assim, há informações confiáveis de que as citocinas pró-inflamatórias promovem apoptose celular (Suematsu et al, 2003), alterações na matriz extracelular (Janczewski et al, 2003), depressão da contratilidade miocárdica (Panas et al, 1998; Sugishita et al, 1999), hipertrofia cardíaca concêntrica (Sivasubramanian et al, 2001; Thomas et al, 2002; van Empel & De Windt, 2004) e cardiomiopatia dilatada (Bozkurt et al, 1998) com o passar do tempo.

A possibilidade do processo de remodelamento miocárdico decorrente da estimulação β-adrenérgica ocorrer por múltiplas vias de sinalização, além das citocinas

TNF-α e IL-6, nos fez investigar outros prováveis gatilhos condicionantes da resposta hipertrófica associada ao ISO.

Fortes evidências tem postulado importante papel do TGF-β_1 como indutor de HM. De fato, este fator de crescimento, conhecido por ser produzido pelos cardiomiócitos e fibroblastos, tem sido mostrado mediar o processo hipertrófico *in vitro* e *in vivo* (Li et al, 1997; Molkentin & Dorn, 2001). A este respeito, Rosenkranz et al (2002) mostraram que camundongos transgênicos, geneticamente modificados para superexpressar TGF-β_1 no miocárdio, demonstraram expressiva hipertrofia cardíaca associada à alteração nos parâmetros de expressão gênica fetal. Especialmente direcionado a nossos objetivos, destaca-se a ligação existente entre atividade β-adrenérgica exacerbada e sinalização hipertrófica via TGF-β_1 (Osadchii, 2007a). Nesta perspectiva, nós confirmamos (*artigo 2*: figura 4C) resultados preliminares da literatura, em que a administração de ISO induziu aumento na expressão gênica do TGF-β_1 (Omura et al, 1994; Boluyt et al, 1995; Masson et al, 1998). Mais especificamente, a descrição de associação positiva entre níveis miocárdicos elevados de RNAm do TGF-β1 e aumento da razão peso cardíaco/peso corporal (Boluyt et al, 1995) intensificaram as opiniões concordantes acerca da participação do TGF-β1 no processo de HM induzida pelo ISO.

A partir de nossas observações de aumento da expressão gênica do TGF-β_1, é presumível que a sinalização hipertrófica intracelular via NF-kB esteja aumentada após estímulo miocárdico com ISO. A validade desta questão emerge do pressuposto que a ligação do TGF-β_1 a receptores de membrana celular promove ativação intracelular da quinase ativadora do TGFβ (TAK1) (Molkentin & Dorn, 2001; Force et al, 2002). A TAK1 leva à ativação do complexo quinase IkK, resultando em degradação das proteínas

citoplasmáticas inibidoras do kB e consequente translocação do fator nuclear kB (NF-kB) para o núcleo celular (Force et al, 2002), onde, sozinho, ou em cooperação com outros fatores de transcrição, induz a expressão de ampla gama de genes envolvidos com o processo hipertrófico (Li et al, 2000; Baldwin, 2001; Hirotani et al, 2002). Além disso, o ISO é conhecido por conceber aumentos na produção de angiotensina II e espécies reativas de oxigênio (Grimm et al, 1999; Ishizawa et al, 2006), fatores sabidamente implicados na HM, via ativação do NF-kB (Force et al, 2002).

Em nosso trabalho, nos valemos do emprego de método imunohistoquímico para caracterizar o possível envolvimento do NF-kB na HM desencadeada pelo ISO. Não se deixe de mencionar que, considerando a ação efetiva do NF-kB somente quando de sua presença no núcleo celular (Baldwin 2001; Hirotani et al, 2002; Hayden & Ghosh, 2004), a avaliação da expressão de núcleos imunopositivos para NF-kB parece ser uma medida válida particularmente interessante. Executando-se este procedimento, nossos resultados são fortemente sugestivos da participação do NF-kB na HM induzida pelo ISO. Assim, a análise da Figura 6G (*artigo 2*) ilustra a existência de maior número de núcleos imuno-positivos para NF-kB nos cardiomiócitos β-adrenergicamente estimulados. Estatisticamente, esta situação ficou configurada quando os animais sedentários tratados com ISO foram comparados aos animais sedentários e treinados que receberam somente óleo de oliva (*artigo 2*: figura 6J). Este é um importante achado e corrobora resultados prévios da literatura. Em camundongos geneticamente modificados para expressar um repressor restrito aos cardiomiócitos do NF-kB, Freund et al (2005) demonstraram que, em resposta a sete dias de tratamento com ISO, a HM foi significativamente atenuada. Em adição, outros dois estudos confirmaram a importância da sinalização NF-kB na HM

induzida pelo ISO. Takemoto et al (1999) e Chandrasekar et al (2004) descreveram que administração de ISO intensificou a atividade ligante ao DNA do NF-kB, um indicativo de seu potencial de efeito na transcrição.

A verificação de nosso primeiro estudo, o efeito anti-hipertrófico do EXF na vigência de acentuada estimulação β-adrenérgica (*artigo 1:* tabela 1), foi novamente demonstrada no segundo artigo (figura 1). Destaque-se a importante relevância clínica destes achados que, conferem ao EXF a capacidade notável para inibir o remodelamento ventricular esquerdo patológico. Os mecanismos participantes desta ação cardioprotetora permanecem para serem esclarecidos em estudos posteriores, contudo, de nossos resultados derivam possíveis explicações. Assim, o EXF foi capaz de abolir o aumento das citocinas pró-inflamatórias TNF-α e IL-6 no miocárdio submetido ao insulto β-adrenérgico. Saliente-se, ainda, a melhora na expressão miocárdica da citocina anti-inflamatória IL-10. Estes efeitos tornaram factual a manutenção da normalidade das relações entre TNF-α/IL10 e IL-6/IL-10 (*artigo 2*: figuras 4B e 5B) nos animais exercitados tratados com ISO. Nesta perspectiva, é esperado que a modulação favorável do balanço entre citocinas pró e anti-inflamatórias promovida pelo EXF possa contribuir para a prevenção do remodelamento miocárdico induzido pelo ISO.

Outro dado destacável de nosso estudo, e passível de contribuir para o efeito anti-hipertrófico do EXF, além da inibição do aumento das citocinas TNF-α e IL-6 no miocárdio, foi a atenuação do conteúdo de RNA mensageiro do TGF-β_1 no miocárdio dos ratos treinados submetidos ao ISO (*artigo 2*: figura 4C). Em adição, na vigência do ISO, apesar de não ser evidenciada redução estatisticamente significante em relação ao grupo sedentário, o grupo de animais previamente treinados mostraram valores inferiores de

positividade nuclear para o NF-kB, a ponto de não diferirem significativamente dos demais grupos do estudo (*artigo 2*: figura 6J). Tais particularidades permitem concordar com a idéia corrente que o EXF pode constituir intervenção eficiente para modulação favorável de fatores de crescimento e/ou fatores de transcrição envolvidos com o processo de HM patológica (Konhilas et al, 2006).

A interpretação dos resultados constantes no *artigo 2,* tabela 1, em especial, os valores da fração de encurtamento (FE) e derivada positiva máxima da pressão desenvolvida do ventrículo esquerdo (+dP/dt), nos levam a considerar que a ação do ISO conduziu à melhora da contratilidade miocárdica e capacitou o ventrículo esquerdo a gerar pressão mais eficientemente. A melhora das variáveis que compõem a regulação da função ejetante do coração é notória em estudos que se valeram de ensaios experimentais *in vivo* ou em preparações de corações isolados para analisar as repercussões do ISO sobre a função cardíaca global (Taylor & White, 1983; Taylor & Tang 1984a, Tang et al, 1987). Realmente, em experiências anteriores do laboratório (Murad & Tucci, 2000), nas quais a função cardíaca global foi avaliada em corações operando isoladamente, a capacidade para gerar pressão foi expressivamente melhorada no ventrículo esquerdo hipertrofiado pelo ISO. Contudo, as informações atuais relativas ao estudo da mecânica miocárdica *in vitro* sugerem que a melhora do estado contrátil do miocárdio associado ao ISO é apenas ilusória. Como ilustrado na Figura 2A/B (*artigo 2*), a ação do ISO conduziu ao prejuízo da capacidade contrátil do miocárdio nos animais sedentários. Estas observações são condizentes com outros estudos da literatura que demonstram redução da função contrátil induzida pelo ISO em amostras musculares isoladas (Hayes et al, 1986; Vassallo et al, 1988; Stein et al, 1996).

A inferência de que ocorre depressão da função mecânica do miocárdio nos animais sedentários com HM decorre, também, de outros achados. Nossos dados caracterizam que a HM despertada pelo ISO conduz a valores elevados de pressão diastólica final do ventrículo esquerdo (*artigo 2:* tabela 1) e a valores reduzidos da taxa de decaimento máxima da tensão desenvolvida (*artigo 2:* figura 2C) e, portanto, configurando um estado deprimido de relaxamento miocárdico.

Em adição, o protocolo experimental *in vitro* possibilitou caracterizar, em preparações de músculos papilares, a sensibilidade dos miofilamentos ao mecanismo de Frank-Starling, isto é, a intensificação da tensão desenvolvida promovida pelo estiramento muscular. Assim, quando se analisaram as retas de estiramento-tensão ativa (*artigo 2*: figura 3) foi evidenciado que os estiramentos musculares acentuaram menos a tensão desenvolvida nos papilares dos animais sedentários submetidos ao ISO. Consequentemente, as inclinações das retas provenientes da relação estiramento muscular e tensão desenvolvida são sugestivas de piora do mecanismo de Frank-Starling. Levando em conta este procedimento, os animais sedentários foram prejudicados pelo ISO.

Estas considerações nos levam a propor que a HM desencadeada pelo ISO é acompanhada por depressão da função miocárdica, que pode ser detectada pelo desempenho de músculos papilares (*artigo 2*: figuras 2 e 3). Vale dizer que nossos dados *in vivo* de melhora dos parâmetros de contratilidade (FE e +dP/dt) nos corações hipertrofiados são compreensíveis pela análise das implicações físicas da hipertrofia. Neste sentido, atentando para o fato de que a hipertrofia concêntrica despertada pelo ISO conduz ao aumento da relação massa/volume (Murad & Tucci, 2000), facilitando, assim, a geração de pressão (Lei de Laplace), é concebível que os valores de FE e +dP/dt

estejam mais elevados no VE hipertrofiado, apesar de sua capacidade para gerar força deprimida. A base para estas considerações provém de estudo anterior de nosso grupo, em que foi observada correlação significante entre a derivada máxima da pressão desenvolvida e relação massa/volume em corações isolados de animais tratados com ISO (Murad & Tucci, 2000). A totalidade de nossos achados permite compreender a impropriedade de considerar, apenas, a FE e +dP/dt como indicadoras de capacidade contrátil miocárdica no modelo de ISO.

É difícil estabelecer com precisão os mecanismos condicionantes da disfunção miocárdica induzida pelo ISO. Contudo, nós dispomos de informações que permitem racionalizar, de maneira fundamentada, sobre o assunto. Nossos resultados evidenciaram que necrose (*artigo 1:* figura 1B) e apoptose (*artigo 1:* figura 3) coexistem no miocárdio submetido ao insulto β-adrenérgico, o que reduz significativamente o número de miofibrilas e, consequentemente, a capacidade contrátil intrínseca do miocárdio. Há a se considerar, ainda, que o acúmulo de colágeno no ventrículo esquerdo (*artigo 1:* figura 1F) pode piorar a distensibilidade e prejudicar a transmissão de força entre os cardiomiócitos em contração (Jalil et al, 1989; Matsubara et al, 2000; Murad & Tucci, 2000; Gallego et al, 2001; Hanft et al, 2008). Considere-se, ainda, a existência de edema, que dificulta a interação miosina-actina por aumentar o espaço interfilamentar (Bragadeesh et al, 2008). Também, aumento da rigidez diastólica do miocárdio esta freqüentemente associada à HM do tipo concêntrica (Vangheluwe et al, 2006). Adicionalmente, há dados da literatura, em nível molecular, demonstrando que a hiperatividade β-adrenérgica induz modificações deletérias na expressão ou atividade de proteínas reguladoras envolvidas com o ciclo de contração e relaxamento do miocárdio.

Assim, a administração de ISO reduziu os conteúdos de AMP cíclico (Stein et al, 1996), SERCA2 (Stein et al, 1996), fosfolambam (Stein et al, 1996) e fosfolambam fosforilada no miocárdio (Nakajima-Takenaka et al, 2009).

Considerando os resultados relacionados com a influência isolada do exercício físico, a literatura tem pontuado o papel do EXF no melhoramento da função miocárdica de ratos idosos (Ascensão et al, 2007; Barmeyer et al, 2009), hipertensos (Morris et al, 2007; Miyachi et al, 2009; Ziada, 2009) e com infarto do miocárdio (Wisløff et al, 2002; Freimann et al, 2005; Leosco et al, 2008; Portes et al, 2009). Nossos resultados ampliam o cenário de atuação protetora do EXF, reportando, pela primeira vez, que o EXF pode prevenir as anormalidades funcionais cardíacas provenientes da ação β-adrenérgica sustentada.

Os estudos da mecânica miocárdica ofereceram informações que permitiram avançar sobre o efeito do EXF. Constatamos que os indicadores de capacidade contrátil (TD e +dT/dt) foram claramente superiores nos ratos treinados, uma propriedade já apontada anteriormente em experimentos que avaliaram os efeitos do EXF sobre a mecânica de músculos papilares (Molé, 1978). A resposta contrátil aos diferentes comprimentos musculares indicou que a funcionalidade do mecanismo de Frank-Starling estava significativamente aprimorada nos animais exercitados. Apesar de nós não termos analisado os possíveis mecanismos responsáveis pelo aprimoramento da contratilidade, destacam-se as já reconhecidas ações do EXF sobre a melhora da sensibilidade dos miofilamentos ao cálcio (Diffee et al, 2001; Wisløff et al, 2001; Diffee & Nagle, 2003a) e o aumento da expressão da cadeia leve da miosina no miocárdio (Diffee & Chung, 2003b).

Uma particularidade notável em nossos dados é que a hiperatividade β-adrenérgica com ISO não foi capaz de inibir o aprimoramento da capacidade contrátil do miocárdio nos ratos exercitados. Os mecanismos participantes desta ação benéfica do EXF permanecem para serem esclarecidos em estudos posteriores, contudo, nossos dados chamam a atenção para algumas circunstâncias que devem ser consideradas: (1) o EXF inibiu a necrose e apoptose no miocárdio injuriado, preservando as estruturas contráteis; (2) nos animais treinados submetidos ao ISO não houve aumento do conteúdo de colágeno no miocárdio, resultando em manutenção das propriedades elásticas e geração de força dos cardiomiócitos.

De uma maneira geral, este estudo experimental é a primeira demonstração de que o EXF, iniciado antes da sobrecarga β-adrenérgica, pode prevenir o remodelamento cardíaco e melhorar a função miocárdica intrínseca. Tendo em conta que o bloqueio β-adrenérgico é uma recomendação padrão para o tratamento da HM e insuficiência cardíaca (Hunt et al, 2005) e que, a dose de ISO por nós utilizada é excessiva em relação à estimulação β-adrenérgica fisiológica ou fisiopatológica habitual, nossos resultados sugerem fortemente que o EXF regular pode constituir intervenção não farmacológica amplamente efetiva no combate aos efeitos adversos da sobrecarga β-adrenérgica.

CONCLUSÕES

1. A sobrecarga beta-adrenérgica com ISO induziu hipertrofia, necrose e apoptose dos cardiomiócitos, acompanhadas por aumento tecidual das citocinas TNFα, IL-6 e TGFβ_1. Soma-se, também, o aumento da expressão nuclear do NF-κB causado pelo ISO;
2. No grupo de animais exercitados houve inibição da hipertrofia cardíaca e prevenção das anormalidades estruturais e ultraestruturais miocárdicas causada pelo isoproterenol;
3. O efeito anti-remodelamento do EXF pode ser devido, em parte, à manutenção tecidual da relação entre citocinas pró e anti-inflamatórias, bem como atenuação da expressão miocárdica do TGFβ_1;
4. O mecanismo que determinou a melhora dos padrões de contratilidade do músculo cardíaco nos ratos exercitados não foi elucidado por este estudo.

REFERENCIAS BIBLIOGRAFICAS

Alderman EL, Harrison DC. Myocardial hypertrophy resulting from low dosage isoproterenol administration in rats. *Proc Soc Exp Biol Med* 1971; **136:**268-70.

Anderson M, Moore D, Larson D. Comparison of isoproterenol and dobutamine in the induction of cardiac hypertrophy and fibrosis. *Perfusion* 2008; **23:**231-5.

Ascensão A, Ferreira R, Magalhães J. Exercise-induced cardioprotection-biochemical, morphological and functional evidence in whole tissue and isolated mitochondria. *Int J Cardiol* 2007; **117:**16-30.

Baldwin KM, Ernest SB, Mullin WJ, Schrader LF, Herrick RE. Exercise capacity and cardiac function of rats with drug-induced cardiac enlargement. *J Appl Physiol* 1982; **52:**591-5.

Baldwin AS. The transcription factor NF-kB and human disease. *J Clin Invest* 2001; **107:**3-6.

Barmeyer A, Müllerleile K, Mortensen K, Meinertz T. Diastolic dysfunction in exercise and its role for exercise capacity. *Heart Fail Rev* 2009; **14:** 125-34.

Batista ML Jr, Santos RV, Oliveira EM, Seelaender MC, Costa Rosa LF. Endurance training restores peritoneal macrophage function in post-MI congestive heart failure rats. *J Appl Physiol* 2007; **102:**2033-9.

Belardinelli R, Georgiou D, Cianci G, Purcaro A. Randomized, controlled trial of long-term moderate exercise training in chronic heart failure: effects on functional capacity, quality of life, and clinical outcome. *Circulation* 1999; **99:** 1173-82.

Benjamin IJ, Jalil JE, Tan LB, Cho K, Weber KT, Clark WA. Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. *Circ Res* 1989; **65:**657-70.

Bertagnolli M, Schenkel PC, Campos C, Mostarda CT, Casarini DE, Belló-Klein A, Irigoyen MC, Rigatto K. Exercise training reduces sympathetic modulation on cardiovascular system and cardiac oxidative stress in spontaneously hypertensive rats. *Am J Hypertens* 2008; **21:**1188-93.

Beznak M, Hacker P. Hemodynamics during the chronic stage of myocardial damage caused by isoproterenol. *Can J Physiol Pharmacol* 1964; **42:**269-74.

Billman GE. Aerobic exercise conditioning: a nonpharmacological antiarrhythmic intervention. *J Appl Physiol* 2002; **92:**446-54.

Bloom S, Cancilla PA. Myocytolis and mitochondrial calcification in rat myocardium after low doses of isoproterenol. *Am J Pathol* 1969; **54:**373-91.

Bloom S, Davis DL. Calcium as mediator of isoproterenol-induced myocardial necrosis. *Am J Pathol* 1972; **69:**459-70.

Bocalini DS, dos Santos L, Serra AJ. Physical exercise improves the functional capacity and quality of life in patients with heart failure. *Clinics (São Paulo)* 2008; **63:**437-42.

Boluyt MO, Long X, Eschenhagen T, Mende U, Schmitz W, Crow MT, Lakatta EG. Isoproterenol infusion induces alterations in expression of hypertrophy-associated genes in rat heart. *Am J Physiol Heart Circ Physiol* 1995; **269:**H638-H47.

Bozkurt B, Kribbs SB, Clubb FJ, Michael LH, Didenko VV, Hornsby PJ, Seta Y, Oral H, Spinale FG Mann DL. Pathophysiologically relevant concentrations of tumor necrosis factor-α promote progressive left ventricular dysfunction and remodeling in rats. *Circulation* 1998; **97:**1382-91.

Brand T, Sharma HS, Schaper W. Expression of nuclear proto-oncogenes in isoproterenol-induced cardiac hypertrophy. *J Mol Cell Cardiol* 1993; **25:**1325-37.

Bragadeesh T, Jayaweera A, Pascotto M, Micari P, Le E, Kramer C, Epstein F, Kaul S. Post-ischemic myocardial dysfunction (stunning) results from myofibrillar edema. *Heart* 2008; **94:**166-77.

Braunwald E. ACE inhibitors - A cornerstone of the treatment of heart failure. *N Engl J Med* 1991; **325:**351-3.

Brodowicz GR, Lamb DR. Exercise training, indomethacin, and isoproterenol-induced myocardial necrosis in the rat. *Basic Res Cardiol* 1991; **86:**40-8.

Brouri F, Hanoun N, Mediani O, Saurini F, Hamon M, Vanhoutte PM, Lechat P. Blockade of beta 1- and desensitization of beta 2-adrenoceptors reduce isoprenaline-induced cardiac fibrosis. *Eur J Pharmacol* 2004; **485:**227-34.

Brum PC, Kosek J, Patterson A, Bernstein D, Kobilka B. Abnormal cardiac function associated with sympathetic nervous system hyperactivity in mice. *Am J Physiol Heart Circ Physiol* 2002; **283:**H1838-H45.

Brunner-La Rocca HP, Esler MD, Jennings GL, Kaye DM. Effect of cardiac sympathetic nervous activity on mode of death in congestive heart failure. *Eur Heart J* 2001; **22:**1136-43.

Chandrasekar B, Marelli-Berg FM, Tone M, Bysani S, Prabhu SD, Murray DR. Beta-adrenergic stimulation induces interleukin-18 expression via beta2-AR, PI3K, Akt, IKK, and NF-kappaB. *Biochem Biophys Res Commun* 2004; **319:**304-11.

Chatteriee K. Congestive heart failure: what should be the initial therapy and why? *Am J Cardiovasc Drugs* 2002; **2:**1-6.

Coats AJ, Adamopoulos S, Radaelli A, McCance A, Meyer TE, Bernardi L, Solda PL, Davey P, Ormerod O, Forfar C, Conway J, Sleight P McCance A, Meyer TE, Bernardi L, Solda PL, Davey P, Ormerod O, Forfar C, Conway J, Sleight P. Controlled trial of physical training in chronic heart failure. Exercise performance, hemodynamics, ventilation, and autonomic function. *Circulation* 1992; **85:**2119-31.

Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, Rector T. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N Engl J Med* 1984; **311:**819-23.

Cornelissen VA, Arnout J, Holvoet P, Fagard RH. Influence of exercise at lower and higher intensity on blood pressure and cardiovascular risk factors at older age. *J Hypertens* 2009; **27:**753-762.

Corrà U, Mezzani A, Giannuzzi P, Tavazzi L. Chronic heart failure-related myopathy and exercise training: a developing therapy for heart failure symptoms. *Curr Probl Cardiol* 2003; **28:**521-47.

Crandall DL, Feirer RP, Griffith DR, Beitz DC. Relative role of caloric restriction and exercise training upon susceptibility to isoproterenol-induced myocardial infarction in male rats. *Am J Clin Nutr* 1981; **34:**841-7.

Darrah MI, Engen RL. Beneficial effects of exercise on l-isoproterenol induced myocardial infarction in male rats. *Med Sci Sports Exerc* 1982; **14:**76-80.

Diffee GM, Seversen EA, Titus MM. Exercise training increases the Ca^{2+} sensitivity of tension in rat cardiac myocytes. *J Appl Physiol* 2001; **91:**309-15.

Diffee GM, Nagle DF. Regional differences in effects of exercise training on contractile and biochemical properties of rat cardiac myocytes. *J Appl Physiol* 2003; **95:**35-42.

Diffee GM, Chung E. Altered single cell force-velocity and power properties in exercise-trained rat myocardium. *J Appl Physiol* 2003; **94:** 1941-48.

Dorn GW. Adrenergic pathways and left ventricular remodeling. *J Card Fail* 2002;

8:S370-3.

Dudnakova TV, Lakomkin VL, Tsyplenkova VG, Shekhonin BV, Shirinskiĭ VP, Kapel'ko VI. Isoproterenol induced changes of protein expression and myocardial ultrastructure. *Kardiologiia* 2002; **42:**57-63.

Ennis IL, Escudero EM, Console GM, Camihort G, Dumm CG, Seidler RW, Hurtado MC, Cingolani HE. Regression of isoproterenol-induced cardiac hypertrophy by Na+/H+ exchanger inhibition. *Hypertension* 2003; **41:**1324-29.

Fan G-C, Yuan Q, Song G, Wang Y, Chen G, Qian J, Zhou X, Lee YJ, Ashraf M, Kranias EG. Small Heat-Shock Protein Hsp20 Attenuates ß-Agonist–Mediated Cardiac Remodeling Through Apoptosis Signal–Regulating Kinase 1. *Circ Res* 2006; **99**:1233-42.

Fleming JW, Wisler PL, Watanabe AM. Signal transduction by G proteins in cardiac tissues. *Circulation* 1992; **85:**420-33.

Fletcher GF, Balady GJ, Amsterdam EA, Chaitman B, Eckel R, Fleg J, Froelicher VF, Leon AS, Piña IL, Rodney R, Simons-Morton DA, Williams MA, Bazzarre T. Exercise standards for testing and training: a statement for healthcare professionals from the American Heart Association. *Circulation* 2001; **104:**1694-740.

Floras JS. Sympathetic activation in human heart failure: diverse mechanisms, therapeutic opportunities. *Acta Physiol Scand* 2003; **177:**391-8.

Force T, Haq S, Kilter H, Michael A. Apoptosis signal-regulating kinase/nuclear factor-kB. A novel signaling pathway regulates cardiomyocyte hypertrophy. *Circulation* 2002; **105:** 402-404.

Fraga R, Franco FG, Roveda F, de Matos LN, Braga AM, Rondon MU, Rotta DR, Brum PC, Barretto AC, Middlekauff HR, Negrão CE. Exercise training reduces sympathetic nerve activity in heart failure patients treated with carvedilol. *Eur J Heart Fail* 2007; **9:**630-6.

Francis GS, Cohn JN, Johnson G, Rector TS, Goldman S, Simon A. Plasma norepinephrine, plasma renin activity, and congestive heart failure. Relations to survival and the effects of therapy in V-HeFT II. The V-HeFT VA Cooperative Studies Group. *Circulation* 1993; **87:**VI40-VI8.

Franklin BA, Kahn JK. Delayed progression or regression of coronary atherosclerosis

with intensive risk factor modification. Effects of diet, drugs, and exercise. *Sports Med* 1996; **22:**306-20.

Frederico MJ, Justo SL, Da Luz G, Da Silva S, Medeiros C, Barbosa VA, Silva LA, Boeck CR, De Pinho RA, De Souza CT. Exercise training provides cardioprotection via a reduction in reactive oxygen species in rats submitted to myocardial infarction induced by isoproterenol. *Free Radic Res* 2009; **11**:1-8.

Freimann S, Scheinowitz M, Yekutiele D, Feinberg MS, Eldar M, Kessler-Icekson G. Prior exercise training improves the outcome of acute myocardial infarction in the rat. Heart structure, function, and gene expression. *J Am Coll Cardiol* 2005; **45:**931-38.

Freimann S, Kessler-Icekson G, Shahar I, Radom-Aizik S, Yitzhaky A, Eldar M, Scheinowitz M. Exercise training alters the molecular response to myocardial infarction. *Med Sci Sports Exerc* 2009; **41:**757-65.

Freund C, Schmidt-Ullrich R, Baurand A, Dunger S, Schneider W, Loser P, El-jamali a, dietz r, scheidereit c, bergmann mw. Requirement of nuclear factor-kappaB in angiotensin II- and isoproterenol-induced cardiac hypertrophy in vivo. *Circulation* 2005; **111:**2319-25.

Gallego M, Espiña L, Vegas L, Echevarria E, Iriarte MM, Casis O. Spironolactone and captopril attenuates isoproterenol-induced cardiac remodelling in rats. *Pharmacol Res* 2001; **44:**311-15.

Gálvez AS, Fiedler JL, Ocaranza MP, Jalil JE, Lavandero S, Díaz-Araya G. Perindopril regulates beta-agonist-induced cardiac apoptosis. *J Cardiovasc Pharmacol* 2005; **46**:255-61.

Garciarena CD, Pinilla OA, Nolly MB, Laguens RP, Escudero EM, Cingolani HE, Ennis IL.Endurance training in the spontaneously hypertensive rat: conversion of pathological into physiological cardiac hypertrophy. *Hypertension* 2009; **53:**708-14.

Gao JP, Chen CX, Wang Y, Lü J, Gu WL. Effect of sodium houttuyfonate on myocardial hypertrophy in mice and rats. *J Pharm Pharmacol* 2009; **61:**677-83.

Gerder AM, Liu Z, Zimmer HG. Changes in nuclear size of cardiac myocytes during the development and progression of hypertrophy in rats. *Cardioscience* 1994; **5**: 203-8.

Giannuzzi P, Mezzani A, Saner H, Björnstad H, Fioretti P, Mendes M, Cohen-Solal A, Dugmore L, Hambrecht R, Hellemans I, McGee H, Perk J, Vanhees L, Veress G;

Working Group on Cardiac Rehabilitation and Exercise Physiology. European Society of Cardiology. Physical activity for primary and secondary prevention. Position paper of the Working Group on Cardiac Rehabilitation and Exercise Physiology of the European Society of Cardiology. *Eur J Cardiovasc Prev Rehabil* 2003; **10:**319-27.

Gibala MJ. High-intensity interval training: a time-efficient strategy for health promotion? *Curr Sports Med Rep* 2007; **6:**211-3.

Goldspink DF, Burniston JG, Ellison GM, Clark WA, Tan LB. Catecholamine-induced apoptosis and necrosis in cardiac and skeletal myocytes of the rat in vivo: the same or separate death pathways? Exp Physiol 2004; **89:**407-16.

Grassi G, Seravalle G, Quarti-Trevano F, Dell'oro R Sympathetic activation in congestive heart failure: evidence, consequences and therapeutic implications. *Curr Vasc Pharmacol* 2009; **7:**137-45.

Greenwood JP, Scott EM, Stoker JB, Mary DA. Hypertensive left ventricular hypertrophy: relation to peripheral sympathetic drive. *J Am Coll Cardiol* 2001; **38:** 1711-17.

Grimm D, Elsner D, Schunkert H, Pfeifer M, Griese D, Bruckschlegel G, Muders F, Riegger GA, Kromer EP. Development of heart failure following isoproterenol administration in the rat: role of the renin–angiotensin system. *Cardiovasc Res* 1998; **37:**91-100.

Grimm D, Holmer S, Riegger GAJ, Kromer EP. Effects of beta-receptor blockade and angiotensin II type I receptor antagonism in isoproterenol-induced heart failure in the rat. *Cardiovasc Pathol* 1999; **8:**315-23.

Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS* 1988; **96:**379-94.

Haft JI. Cardiovascular injury induced by sympathetic catecholamines. *Prog Cardiovasc Dis* 1974; **17:**73-86.

Hambrecht R, Gielen S, Schuler G. Physical training as an adjunct therapy in patients with congestive heart failure: patient selection, training protocols, results, and future directions. *Curr Cardiol Rep* 1999; **1:**38-46.

Hambrecht R, Gielen S, Linke A, Fiehn E, Yu J, Walther C, Schoene N, Schuler G. Effects of exercise training on left ventricular function and peripheral resistance in patients with chronic heart failure: A randomized trial. *JAMA* 2000; **283:**3095-101.

Handforth CP. Isoproterenol-induced myocardial infarction in animals. *Arch Pathol* 1962; **73:**161-5.

Hanft LM, Korte FS, MacDonald KS. Cardiac function and modulation of sarcomeric function by length. *Cardiovasc Res* 2008; **77:**627-36.

Hayden MS, Ghosh. Signaling to NF-κB. *Genes Dev* 2004; **18:**2195-2224.

Hayes JS, Wyss VL, Schenck KS, Cohen ML. Effects of prolonged isoproterenol infusion on cardiac and vascular responses to adrenoceptor agonists. *J Pharmacol Exp Ther* 1986; **237:**757-63.

Hill JA, Olson EN. Cardiac Plasticity. *N Engl J Med* 2008; **358:**1370-80.

Hirotani S, Otsu K, Nishida K, Higuchi Y, Morita T, Nakayama H, Yamaguchi O, Mano T, Ueno H, Tada M, Hori M. Involvement of nuclear factor-kB and apoptosis signal-regulating kinase 1 in G-protein-coupled receptor agonist-induced cardiomyocyte hypertrophy. *Circulation* 2002; **105:** 509-15.

Hoehner CM, Soares J, Perez DP, Ribeiro IC, Joshu CE, Pratt M, Legetic BD, Malta DC, Matsudo VR, Ramos LR, Simões EJ, Brownson RC. Physical activity interventions in Latin America: a systematic review. *Am J Prev Med* 2008; **34:**224-33.

Hunt SA, Abraham WT, Chin MH, et al. ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001Guidelines for the Evaluation and Management of Heart Failure). *Circulation* 2005; **112:**e154-235.

Ishizawa M, Mizushige K, Noma T, Namba T, Guo P, Murakami K, Tsuji T, Miyatake A, Ohmori K, Kohno M. An antioxidant treatment potentially protects myocardial energy metabolism by regulating uncoupling protein 2 expression in a chronic β-adrenergic stimulation rat model. *Life Sci* 2006; **78:**2974-82.

Jalil JE, Doering CW, Janicki JS, Pick R, Shroff SG, Weber KT. Fibrillar collagen and myocardial stiffness in the intact hypertrophied rat left ventricle. *Circ Res* 1989; **64:**1041-50.

Janczewski AM, Kadokami T, Lemster B, Frye CS, MacTiernan CF, Feldman AM. Morphological and functional changes in cardiac myocytes isolated from mice overexpressing TNF-alpha. *Am J Physiol Heart Circ Physiol* 2003; **284:**H960-69.

Junqueira LCU, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J* 1979; **11:**442-55.

Kannel WB, Gordon T, Offutt D. Left ventricular hypertrophy by electrocardiogram. Prevalences, incidence, and mortality in the Framinghan study. *Ann Intern Med* 1969; **71:**89-105.

Kannel WB, Dannenberg AL, Levy D. Population implications of eletrocardiographic left ventricular hypertrophy. *Am J Cardiol* 1987a; **60:**851-931.

Kannel WB, Levy D, Cupples LA. Left ventricular hypertrophy and risk of cardiac failure: insights from the Framingham study. *J Cardiovasc Pharmacol* 1987b; **10:**S135-S40.

Kaye DM, Lefkovits J, Jennings GL, Bergin P, Broughton A, Esler MD. Adverse consequences of high sympathetic nervous activity in the failing human heart. *J Am Coll Cardiol* 1995; **26:**1257-63.

Kazachenko AA, Okovityĭ SV, Kulikov AN, Gustaĭnis KR, Nagornyĭ MB, Shulenin SN, Erokhina IL, Emel'ianova OI. Comparative characteristics of some pharmacological models of chronic heart failure. *Eksp Klin Farmakol* 2008; **71:**16-9.

Kawamura T, Yoshida K, Sugawara A, Nagasaka M, Mori N, Takeuchi K, Kohzuki M. Impact of exercise and angiotensin converting enzyme inhibition on tumor necrosis factor-alpha and leptin in fructose-fed hypertensive rats. *Hypertens Res* 2002; **25:** 919-26.

Kemi OJ, Ellingsen ø, Ceci M, Grimaldi S, Smith GL, Condorelli G, Wisløff U. Aerobic interval training enhances cardiomyocyte contractility and Ca2+ cycling by phosphorylation of CaMKII and Thr-17 of phospholamban. *J Mol Cell Cardiol* 2007; **43:**354-61.

Kiilavuori K, Näveri H, Leinonen H, Härkönen M. The effect of physical training on hormonal status and exceptional hormonal response in patients with chronic congestive heart failure. *Eur Heart J* 1999; **20:**456-64.

Konhilas JP, Watson PA, Maass A, Boucek DM, Horn T, Stauffer BL, Luckey SW, Rosenberg P, Leineand LA. Exercise can prevent and reverse the severity of hypertrophic cardiomyopathy. *Circ Res* 2006; **98:** 540-48.

Krenek P, Kmecova J, Kucerova D, Bajuszova Z, Musil P, Gazova A, Ochodnicky P, Klimas J, Kyselovic J. Isoproterenol-induced heart failure in the rat is associated with nitric oxide-dependent functional alterations of cardiac function. *Eur J Heart Fail* 2009; **11:**140-6.

Lee IM, Skerrett PJ. Physical activity and all-cause mortality: what is the dose-response relation. *Med Sci Sports Exerc* 2001; **33:**S459-S71.

Leenen FH, White R, Yuan B. Isoproterenol-induced cardiac hypertrophy: role of circulatory versus cardiac renin-angiotensin system. *Am J Physiol Heart Circ Physiol* 2001; **281:**H2410-H6.

Lennon SL, Quindry JC, Frenchi JP, Kin S, Mehta JL, Powers SK. Exercise and myocardial tolerance to ischemia-reperfusion. *Acta Physiol Scand* 2004; **182:**161-9.

Leosco D, Rengo G, Laccarino G, Golino L, Marchese M, Fortunato F, Zincarelli C, Sanzari E, Ciccarelli M, Galasso G, Altobelli GG, Conti V, Matrone G, Cimini V, Ferrara N, Filippelli A, Koch WJ, Rengo F. Exercise promotes angiogenesis and improves beta-adrenergic receptor signalling in the post-ischaemic failing rat heart. *Cardiovasc Res* 2008; **78:**385-94.

Le Page C, Noirez P, Courty J, Riou B, Swynghedauw B, Besse S. Exercise training improves functional post-ischemic recovery in senescent heart. *Exp Gerontol* 2009; **44:**177-82.

Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; **322:**1561-6.

Li RK, Mickle DA, Weisel RD, Merante F, Luss H, Rao V, Christakis GT, Williams WG. Overexpression of transforming growth factor-β1 and insulin-like growth factor-

I in patients with idiopathic hypertrophic cardiomyopathy. *Circulation* 1997; **96:**874-81.

Li R, Pei H, Watson DK. Regulation of Ets functions by protein-protein interactions. *Oncogene* 2000; **19:**6514-23.

Li Z, Tran TT, Ma JY, O´Young G, Kapoun AM, Chakravarty S, Dugar S, Schreiner G, Protter AA. p38 alpha mitogen-activated protein kinase inhibition improves cardiac function and reduces myocardial damage in isoproterenol-induced acute myocardial injury in rats. *J Cardiovasc Pharmacol* 2004; **44**:486-92.

Linck B, Bokník P, Baba HA, Eschenhagen T, Haverkamp U, Jäckel E, Jones LR, Kirchhefer U, Knapp J, Läer S, Müller FU, Schmitz W, Scholz H, Syska A, Vahlensieck U, Neumann J. Long-term beta adrenoceptor-mediated alteration in contractility and expression of phospholamban and sarcoplasmic reticulum Ca^{++}-ATPase in mammalian ventricle. *J Pharmacol Exp Thre* 1998; **286:**531-38.

Lips DJ, deWindt LJ, van Kraaij DJ, Doevendans PA. Molecular determinants of myocardial hypertrophy and failure: alternative pathways for beneficial and maladaptive hypertrophy. *Eur Heart J* 2003; **24:**883-96.

Liu B, Li W, Li Y, Wang Z, Li H, Liu P, Fu J. Protective effects of N-acetylcysteine in isoproterenol-induced myocardium injury in rats. *Mol Biol Rep* 2009; **36:**761-5.

Ljubuncić P, Mujić F, Winterhalter M, Huković N, Vidović Z, Duricić S. Isoproterenol toxicity in the myocardium in an experiment. *Arh Hig Rada Toksikol* 1992a; **43:**11-20.

Ljubuncić P, Stojiljković MP, Winterhalter-Jadrić M, Vidović Z, Mujić F. Efficacy of pretreatment with adrenergic beta-receptor blockers in the prevention of cardiotoxic effects of isoprenaline in rats. *Vojnosanit Pregl* 1992b; **49:**297-304.

Ljubuncić P, Stojiljković MP, Winterhalter-Jadrić M, Mujić F, Vidović Z. The effect of calcium antagonists on the cardiotoxic effects of isoprenaline in rats. *Vojnosanit Pregl* 1992c; **49:**305-11.

Ljubuncić P, Mujić F, Stojiljković MP, Vinterhalter-Jadrić M. The effect of preliminary administration of dimethylsulfoxide on the development of experimental myocardial necrosis in rats treated with isoprenaline. *Srp Arh Celok Lek* 1991; **119:**238-42.

Mann DL. Basic mechanisms of disease progression in the failing heart: the role of excessive adrenergic drive. *Prog Cardiovasc Dis* 1998; **41:**1-8.

Masson S, Arosio B, LuvarÀ G, Gagliano N, Fiordaliso F, Santambrogio D, Vergani C, Latini R, Annoni G. Remodelling of cardiac extracellular matrix during beta-adrenergic stimulation: upregulation of SPARC in the myocardium of adult rats. *J Mol Cell Cardiol* 1998; **30:**1505-14.

Matsubara LS, Matsubara BB, Okoshi MP, Cicogna AC, Janicki JS. Alterations in myocardial collagen content affect rat papillary muscle function. *Am J Physiol Heart Circ Physiol* 2000; **279:**H1534-H39.

Mechelen WV. A physically active lifestyle-public health's best buy? *Br J Sports Med* 1997; **31:**264-65.

Meng D, Feng L, Chen XJ, Yang D, Zhang JN. Trimetazidine improved Ca2+ handling in isoprenaline-mediated myocardial injury of rats. *Exp Physiol* 2006; **91:** 591-601.

Milei J, Nunez RG, Rapaport M. Pathogenesis of isoproterenol-induced myocardial lesions: its relation to human `coagulative myocytolysis'. *Cardiology* 1978; **63:**139-51.

Milei J, Nunez RG, Bolomo NJ. Isoproterenol-induced 45ca uptake into myocardium of rats. *Res Exp Med (Berl)* 1979; **176:**117-21.

Miyachi M, Yazawa H, Furukawa M, Tsuboi K, Ohtake M, Nishizawa T, Hashimoto K, Yokoi T, Kojima T, Murate T, Yokota M, Murohara T, Koike Y, Nagata K. Exercise training alters left ventricular geometry and attenuates heart failure in dahl salt-sensitive hypertensive rats. *Hypertension* 2009; **53:**701-7.

Moholdt T, Wisløff U, Nilsen TI, Slørdahl SA. Physical activity and mortality in men and women with coronary heart disease: a prospective population-based cohort study in Norway (the HUNT study). *Eur J Cardiovasc Prev Rehabil* 2008; **15:**639-45.

Molé PA. Increased contractile potencial of papillary muscles from exercise-trained rat hearts. *Am J Physiol Heart Circ Physiol* 1978; **3:**H421-H25.

Molkentin JD, Dorn GW. Cytoplasmic signaling pathways that regulate cardiac hypertrophy. *Ann Rev Physiol* 2001; **63:** 391-426.

Morgan HE, Baker KM. Cardiac hypertrophy. Mechanical, neural, and endocrine dependence. *Circulation* 1991; **83:**13-25.

Morris RT, Fine DM, Lees SJ, Booth FW, Link CD, Ferrario CM, Stump CS, Sowers JR. Exercise training prevents development of cardiac contractile dysfunction in hypertensive TG (mREN-2)27 rats. *J Am Soc Hypertens* 2007; **1:** 393-99.

Murad N, Tucci PJF. Isoproterenol-induced hypertrophy may result in distinct left ventricular changes. *Clin Exp Pharmacol Physiol* 2000; **27:**352-57.

Murray RM, Prabhu SD, Chandrasekar B. Chronic β-adrenergic stimulation induces myocardial proinflammatory cytokines expression. *Circulation* 2000; **101**:2338-41.

Myers J. Exercise and cardiovascular health. *Circulation* 2003; **107:**e2-e5.

Nagano M, Higaki J, Nakamura F, Higashimori K, Nagano N, Mikami H, Ogihara T. Role of cardiac angiotensin II in isoproterenol-induced left ventricular hypertrophy. *Hypertension* 1992; **19:**708-12.

NegrãCE, Middlekauff HR. Adaptations in autonomic function during exercise training in heart failure. *Heart Fail Rev* 2008; **13:**51-60.

Nakajima-Takenaka C, Zhang GX, Obata K, Tohne K, Matsuyoshi H, Nagai Y, Nishiyama A, Takaki M. Left ventricular function of isoproterenol-induced hypertrophied rat hearts perfused with blood: mechanical work and energetics. *Am J Physiol Heart Circ Physiol* 2009 (In press).

Okuda N, Hayashi T, Mori T, Inamoto S, Okabe M, Mieno S, Horimoto H, Kitaura Y. Nifedipine enhances the cardioprotective effect of an angiotensin-II receptor blocker in an experimental animal model of heart failure. *Hypertens Res* 2005; **28:**431-8.

Oliveira EM, Krieger JE. Chronic β-adrenoreceptor stimulation and cardiac hypertrophy with no induction of circulating rennin. *Eur J Pharmacol* 2005; **520:** 135-41.

Omura T, Kim S, Takeuchi K, Iwao H, Takeda T. Transforming growth factor beta 1 and extracellular matrix gene expression in isoprenaline induced cardiac hypertrophy: effects of inhibition of the renin-angiotensin system. *Cardiovasc Res* 1994; **28:**1835-42.

Osadchii OE. Cardiac hypertrophy induced by sustained beta-adrenoreceptor activation: pathophysiological aspects. *Heart Fail Rev* 2007a; **12:**66-86.

Osadchii OE, Woodiwiss AJ, Deftereos D, Norton GR. Temporal changes in myocardial adrenergic regulation with the progression to pump dysfunction after chronic *β*-adrenoreceptor activation in rats. *Pflugers Arch - Eur J Physiol* 2007b; **455:** 251-60.

Osadchii OE, Woodiwiss A, Alves N, Norton G. Mechanisms of preserved baseline cardiac systolic function in rats with adrenergic inotropic downregulation. *Life Sci* 2005; **78:**366-75.

Oudit GY, Crackower MA, Eriksson U, Sarao R, Kozieradzki I, Sasaki T, Irie-Sasaki J, Gidrewicz D, Rybin VO, Wada T, Steinberg SF, Backx PH, Penninger JM. Phosphoinositide 3-kinase gamma-deficient mice are protected from isoproterenol-induced heart failure. *Circulation* 2003; **108:**2147-52.

Owen KL, Pretorius L, McMullen JR. The protective effects of exercise and phosphoinositide 3-kinase (p110alpha) in the failing heart. *Clin Sci (Lond)* 2009; **116:**365-75.

Packer M. Neurohormonal interactions and adaptations in congestive heart failure. *Circulation* 1988; **77:**721-30.

Panas D, Khadour FH, Szabó C, Schulz R. Proinflammatory cytokines depress cardiac efficiency by a nitric oxide-depedent mechanism. *Am J Physiol Heart Circ Physiol* 1998; **275:**H1016-H23.

Passino C, Severino S, Poletti R, Piepoli MF, Mammini C, Clerico A, Gabutti A, Nassi G, Emdin M. Aerobic training decreases B-type natriuretic peptide expression and adrenergicactivation in patients with heart failure. *J Am Coll Cardiol* 2006; **47:**1835-9.

Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, Buchner D, Ettinger W, Heath GW, King AC, et al. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA* 1995; **273:**402-7.

Paterick TE, Fletcher GF. Endothelial function and cardiovascular prevention: role of blood lipids, exercise, and other risk factors. *Cardiol Rev* 2001; **9:**282-6.

Peterson JA. Get moving! Physical activity counseling in primary care. *J Am Acad Nurse Pract* 2007; **19:**349-57.

Portes LA, Tucci PJ. Swim training attenuates myocardial remodeling and the pulmonary congestion in wistar rats with secondary heart failure to myocardial infarction. *Arq Bras Cardiol* 2006; **87:**54-9.

Portes LA, Saraiva RM, Dos Santos AA, Tucci PJ. Swimming training attenuates remodeling, contractile dysfunction and congestive heart failure in rats with moderate and large myocardial infarctions. *Clin Exp Pharmacol Physiol* 2009; **36:** 394-99.

Prasad DS, Das BC. Physical inactivity: a cardiovascular risk factor. *Indian J Med Sci* 2009; **63:**33-42.

Prabhu S, Jainu M, Sabitha KE. Effect of mangiferin on mithocondrial energy production in experimentally induced myocardial infarcted rats. *Vascul Pharmacol* 2006; **44:**519-25.

Quindry J, French J, Hamilton K, Lee Y, Mehta JL, Powers S. Exercise training provides cardioprotection against ischemia-reperfusion induced apoptosis in young and old animals. *Exp Gerontol* 2005; **40:**416-25.

Rathore N, John S, Kale M, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. *Pharmacol Res* 1998; **38:**297-303.

Riggs CE Jr, Landiss CW, Jessup GT, Bonner HW. Effects of exercise on the severity of isoproterenol-induced myocardial infarction. *Med Sci Sports* 1977; **9:**83-7.

Rona G, Chappel CI, Balazs T, Gaudry R. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. *AMA Arch Pathol* 1959; **67:**443-55.

Rona G, Chappel C, Kahn DS. The significance of factors modifying the development of isoproterenol-induced myocardial necrosis. *Am Heart J* 1963; **66:**389-95.

Rosenkranz S, Flesch M, Amann K, Haeuseler C, Kilter H, Seeland U, Schlüter K-D, Böhm M. Alterations of β-adrenergic signaling and cardiac hypertrophy in transgenic mice overexpression TGF- β_1. *Am J Physiol Heart Circ Physiol* 2002; **283:**H1253-H62.

Roveda F, Middlekauff HR, Rondon MU, Reis SF, Souza M, Nastari L, Barretto AC, Krieger EM, Negrão CE. The effects of exercise training on sympathetic neural activation in advanced heart failure: a randomized controlled trial. *J Am Coll Cardiol* 2003; **42:**854-60.

Saadane N, Alpert L, Chalifour LE. Altered molecular response to adrenoreceptor-induced cardiac hypertrophy in Egr-1- deficient mice. *Am J Physiol Heart Circ Physiol* 2000; **278:**H796-H805.

Saito S, Hiroi Y, Zou Y, Aikawa R, Toko H, Shibasaki F, Yazaki Y, Nagai R, Komuro I. β-adrenergic pathway induced apoptosis through calcineurin activation in cardiac myocytes. *J Biol Chem* 2000; 275; **44:**34528-33.

Schlaich MP, Kaye DM, Lambert E, Sommerville M, Socratous F, Esler MD. Relation between cardiac sympathetic activity and hypertensive left ventricular hypertrophy. *Circulation* 2003; **108:**560-5.

Schumacher A, Khojeini E, Larson D. ECHO parameters of diastolic dysfunction. *Perfusion* 2008; **23:**291-6.

Serra AJ, Higuchi ML, Ihara SS, Antônio EL, Santos MHH, Bombig MTN, Tucci PJF. Exercise training prevents β-adrenergic hyperactivity-induced myocardial hypertrophy and lesions. *Eur J Heart Failure* 2008; **10:**534-39.

Shizukuda Y, Buttrick PM, Geenen DL, Borczuk AC, Kitsis RN, Sonnenblick EH. Beta-adrenergic stimulation causes cardiocyte apoptosis: influence of tachycardia and hypertrophy. *Am J Physiol Heart Circ Physiol* 1998; **75:**H961-H8.

Sivasubramanian N, Coker ML, Kurrelmeyer KM, MacLellan WR, DeMayo FJ, Spinale FG, MANN DL. Left ventricular remodeling in transgenic mice with cardiac restricted overexpression of tumor necrosis factor. *Circulation* 2001; **104:**826-31.

Stanton HC, Bowman Z. Studies on isoproterenol-induced cardiomegaly in rats. *Proc West Pharmacol Soc* 1967; **10:**87-9.

Stein B, BarTel S, Kirchhefer U, Kokott S, Krause EG, Neumann J, Schmitz W, Scholz H. Relation between contractile function and regulatory cardiac protein in hypertrophied hearts. *Am J Physiol Heart Circ Physiol* 1996; **270:** H2021-H28.

Strand AH, Gudmundsdottir H, Os I, Smith G, Westheim AS, Bjornerheim R, Kjeldsen SE. Arterial plasma noradrenaline predicts left ventricular mass independently of blood pressure and body build in men who develop hypertension over 20 years. *J Hypertens* 2006; **24:**905-13.

Suematsu N, Tsutsui H, Wen J, Kang D, Ikeuchi M, Ide T, Hayashidani S, Shiomi T, Kubota T, Hamasaki N, Takeshita A. Oxidative stress mediates tumor necrosis factor-

α-induced mitochondrial DNA damage and dysfunction in cardiac myocytes. *Circulation* 2003; **107:**1418-23.

Sugden PH, Bogoyevitch MA. Intracelular signalling through protein kinases in the heart. *Cardiovasc Res* 1995; **30:**478-92.

Sugishita K, Kinugawa K-I, Shimizu T, Harada K, Matsui H, Takahashi T, Serizawa T, Kohmoto O. Celullar basis for the acute inhibitory effects of IL-6 and TNF-α on excitation-contraction coupling. *J Mol Cell Cardiol* 1999; **31:**1457-67.

Tajuddin M, Ahmad M, Tariq M. Effect of conditioning on myocardial metabolism after myocardial infarction. *Recent Adv Stud Cardiac Struct Metab* 1975; **10:**561-8.

Takemoto Y, Yoshiyama M, Takeuchi K, Omura T, Komatsu R, Izumi Y, kim s, yoshikawa j. Increased JNK, AP-1 and NF-κB DNA binding activities in isoproterenol-induced cardiac remodeling. *J Mol Cell Cardiol* 1999; **31:**2017-30.

Tanaka H. Swimming exercise: impact of aquatic exercise on cardiovascular health. *Sports Med* 2009; **39:**377-87.

Tang Q, Taylor PB, Helbing RK. Catecholamine induced cardiac hypertrophy. *Can J Cardiol* 1987; **3:**311-6.

Tang L, Taylor PB. Altered contractile function in isoproterenol-induced hypertrophy rat heart. *J Hypertens* 1996; **14:**751-7.

Taylor PB, Tang Q. Development of isoproterenol-induced cardiac hypertrophy. *Can J Physiol Pharmacol* 1984a; **62:**384-9.

Tang Q, Taylor PB. Regression of isoproterenol-induced cardiac hypertrophy. *Can J Physiol Pharmacol* 1984b; **62:**1141-6.

Taylor PB, White DM. Isoproterenol induced changes in myocardial nucleic acids. *Res Commun Chem Pathol Pharmacol* 1983; **40:**551-4.

Taylor PB, Helbing RK, Rourke S, Churchill D. Effect of catecholamine-induced cardiac hypertrophy on the force-interval relationship. *Can J Physiol Pharmacol* 1989; **67:**40-6.

Taylor PB, Swanson CT, Draisey TF. Quantitative histological changes in isoproterenol induced cardiac growth. *Res Commun Chem Pathol Pharmacol* 1977; **17**:341-44.

Teerlink JR, Pfeffer JM, Pfeffer MA. Progressive ventricular remodeling in response to diffuse isoproterenol-induced myocardial necrosis in rats. *Circ Res* 1994; **75:**105-13.

Thomas F, Haq S, Kilter H, Michael A. Apoptosis signal-regulating kinase/nuclear factor-kb. A novel signaling pathway regulates cardiomycyte hypertrophy. *Circulation* 2002; **105:**402-4.

Tomita H, Nazmy M, Kajimoto K, Yehia G, Molina CA, Sadoshima J. Inducible cAMP early repressor (ICER) is a negative-feedback regulator of cardiac hypertrophy and an important mediator of cardiac myocyte apoptosis in response to beta-adrenergic receptor stimulation. *Circ Res* 2003; **93**:12-22.

Tyni-Lenné R, Dencker K, Gordon A, Jansson E, Sylvén C. Comprehensive local muscle training increases aerobic working capacity and quality of life and decreases neurohormonal activation in patients with chronic heart failure. *Eur J Heart Fail* 2001; **3:**47-52.

van Empel VP, De Windt LJ. Myocyte hypertrophy and apoptosis: a balancing act. *Cardiovasc Res* 2004; **63:**487-99.

Vangheluwe P, Tjwa M, Van Den Bergh A, Louch WE, Beullens M, Dode L, Carmeliet P, Kranias E, Herijgers P, Sipido KR, Raeymaekers L, Wuytack F. A Serca 2 pump with an increased Ca^{2+} affinity can lead to severe cardiac hypertrophy, stress intolerance and reduced life span. *J Mol Cell Cardiol* 2006; **41:**308-17.

Vassallo DV, Vasquez EC, Cabral AM. Contractile performance of papillary muscles of renovascular hypertensive and isoproterenol-pretreated rats. *Pharmacol Res Commun* 1988; **20:**61-72.

Vona M, Codeluppi GM, Iannino T, Ferrari E, Bogousslavsky J, von Segesser LK. Effects of different types of exercise training followed by detraining on endothelium-dependent dilation in patients with recent myocardial infarction. *Circulation* 2009; **119:**1601-8.

Yeager JC, Whitehurst ME. Verapamil prevents isoproterenol induced cardiac failure in the rat. Life Sci 1982; **30**:299-306.

Zanchi NE, Bechara LR, Tanaka LY, Debbas V, Bartholomeu T, Ramires PR. Moderate exercise training decreases aortic superoxide production in myocardial infarcted rats. *Eur J Appl Physiol* 2008; **104:**1045-52.

Zhang LQ, Zhang XQ, Musch TI, Moore RL, Cheung JY. Sprint training restores normal contractility in postinfarction rat myocytes. *J Appl Physiol* 2000a; **89:**1099-105.

Zhang LQ, Zhang XQ, Ng YC, Rothblum LI, Musch TI, Moore RL, Cheung JY. Sprint training normalizes Ca(2+) transients and SR function in postinfarction rat myocytes. *J Appl Physiol* 2000b; **89:**38-46.

Zhang GX, Kimura S, Nishiyama A, Shokoji T, Rahman M, Yao L, Nagai Y, Fujisawa Y, Miyatake A, Abe Y. Cardiac oxidative stress in acute and chronic isoproterenol-infused rats. *Cardiovasc Res* 2005; **65:**230-38.

Zhou B, Wu L-J, Li L-H, Tashiro S-I, Onodera S, Uchiumi F, Ikejima T. Silibinin protects against isoproterenol-Induced rat cardiac myocyte injury through mitochondrial pathway after up-regulation of SIRT1. *J Pharmacol Sci* 2006; **102:**387-95.

Ziada AM. Additional salutary effects of the combination of exercise training and an angiotensin-converting enzyme inhibitor on the left ventricular function of spontaneously hypertensive rats. *J Hypertens* 2009; **27:**1309-16.

Zierhut W, Zimmer HG. Significance of myocardial alpha- and beta- adrenoceptors in cathecolamine-induced cardiac hypertrophy. *Circ Res* 1989; **65:**1417-25.

Zou Y, Komuro I, Yamazaki T, Kudoh S, Uozumi H, Kadowaki T, Yazaki Y. Both G_s and G_i proteins are critically involved in isoproterenol-induced cardiomyocyte hypertrophy. *J Biol Chem* 1999; **274:**9760-70.

Zou Y, Yao A, Zhu W, Kudoh S, Hiroi Y, Shimoyama M, Uozumi H, Kohmoto O, Takahashi T, Shibasaki F, Nagai R, Yazaki Y, Komuri I. Isoproterenol activates extracellular signal-regulated protein kinases in cardiomyocytes through calcineurin. *Circulation* 2001; **104**:102-8.

Yap TL, Davis LS. Physical activity: the science of health promotion through tailored messages. *Rehabil Nurs* 2008; **33:**55-62.

Williams MA, Haskell WL, Ades PA, Amsterdam EA, Bittner V, Franklin BA, Gulanick M, Laing ST, Stewart KJ; American Heart Association Council on Clinical Cardiology; American Heart Association Council on Nutrition, Physical Activity, and Metabolism. Resistance exercise in individuals with and without cardiovascular disease: 2007 update: a scientific statement from the American Heart Association Council on Clinical Cardiology and Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2007; **116:**572-84.

Wisløff U, Loennechen JP, Currie S, Smith GL, Ellingsen Ø. Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca^{2+} sensitivity and SERCA-2 in rat after myocardial infarction. *Cardiovasc Res* 2002; **54:**162-74.

Wisløff U, Loennechen JP, Falck G, Beisvag V, Currie S, Smith G, Ellingsen Ø. Increased contractility and calcium sensitivity in cardiac myocytes isolated from endurance trained rats. *Cardiovasc Res* 2001; **50:**495-508.

Woodiwiss AJ, Tsotetsi OJ, Sprott S, Lancaster EJ, Mela T, Chung ES, Meyer TE, Norton GR. Reduction in myocardial collagen cross-linking parallels left ventricular dilatation in rat models of systolic chamber dysfunction. *Circulation* 2001; **103:**155-60.