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Exercise training, Genetics and Heart Failure

The effect of exercise training and gene variants on left ventricular function and exercise tolerance in heart failure patients

Alberto Jorge Carvalho Alves

Dissertação apresentada com vista à obtenção do grau de Doutor no âmbito do Curso de Doutoramento em Atividade Física e Saúde, organizado pelo Centro de Actividade Física, Saúde e Lazer (CIAFEL) da Faculdade de Desporto da Universidade do Porto, nos termos do Decreto-lei 74/2006 de 24 de Março.

Supervisores: José Oliveira, PhD

Michael Sagiv, PhD

Porto 2011

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To my mom, an example of dedication, inner strength and hard work

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Resumo

O presente trabalho teve como objectivo a avaliação dos efeitos do exercício crónico, de variantes genéticas e da interacção entre ambos na função e estrutura do ventrículo esquerdo e na tolerância ao exercício físico, em pacientes com insuficiência cardíaca. Pacientes com insuficiência cardíaca e fracção de ejecção do ventrículo esquerdo (FEVE) moderada a severa, ligeira e preservada foram divididos aleatoriamente em dois grupos com uma razão de 2 pacientes no grupo de exercício para 1 paciente no grupo beneficiando apenas do tratamento médico habitual. O programa de treino consistiu em 3 sessões semanais de exercício aeróbio durante 6 meses. A função e as dimensões do ventrículo esquerdo bem como a tolerância ao exercício físico foram avaliadas antes e após 6 meses de cada uma das intervenções. As variantes genéticas *ADRB1* Arg389Gly (rs1801253), *GNAS* -1211 G/A (rs6123837), *GNAS* 2291 C/T (rs6026584), *PPARGC1A* Gly482Ser (rs8192678), *PPARGC1A* 2962 G/A (rs6821591), *PPARA* Leu162Val (rs1800206), *PPARA* Intron 7 C/G (rs4253778), *PPARD* 294 C/T (rs2016520) and *NRF2* A/C (rs12594956) foram avaliadas através da análise de polimorfismos dos fragmentos de restrição do DNA genómico. O programa de exercício físico melhorou significativamente a função do ventrículo esquerdo e a tolerância ao exercício em todos os grupos de insuficiência cardíaca. Adicionalmente, o exercício físico reduziu significativamente os diâmetros ventriculares no grupo de pacientes com disfunção sistólica moderada a severa, mas não de forma consistente nos grupos de pacientes com disfunção sistólica ligeira e função sistólica preservada. A análise da função ventricular entre diferentes genótipos revelou que o tempo de desaceleração diastólica antes da intervenção era mais prolongado nos portadores do alelo *GNAS* -1211G comparativamente aos homozigóticos para o alelo *GNAS* -1211A ($P<0.05$). O exercício físico diminuiu o tempo de desaceleração diastólica nos portadores do alelo *GNAS* -1211G ($P<0.05$), mas não nos homozigóticos para o alelo *GNAS* -1211A. De igual forma, os pacientes homozigóticos para a variante *PPARGC1A* Ser482 exibiram uma FEVE significativamente mais reduzida do que os pacientes heterozigóticos ($P=0.027$) e tendencialmente mais reduzida do que aquela observada nos homozigóticos para a variante *PPARGC1A* Gly482 ($P=0.059$). O exercício regular promoveu um aumento mais pronunciado da FEVE nos pacientes homozigóticos para a variante *PPARGC1A* Ser482 comparativamente aos pacientes heterozigóticos ($P=0.037$). Contudo, não foram encontradas diferenças entre homozigóticos para a variante Ser482 e homozigóticos para a variante Gly482 ($P=0.170$). Adicionalmente, o aumento da tolerância ao exercício induzido pelo programa de treino foi significativamente maior em pacientes com dois alelos de *ADRB1* 389Gly do que em pacientes com dois alelos de 389Arg ($P=0.04$). Para além disso, o aumento da tolerância ao exercício físico promovido pelo programa de treino foi maior em pacientes portadores do alelo *PPARA* 162Val do que em pacientes homozigóticos para o alelo 162Leu ($P=0.032$). Finalmente, o programa de treino aumentou a tolerância ao exercício em pacientes portadores do alelo A do gene *NRF2* ($P<0.05$), mas não em homozigóticos para o alelo G. Os presentes resultados indicam que o exercício crónico melhora a função do ventrículo esquerdo e a tolerância ao exercício em todos os grupos de insuficiência cardíaca. Para além disso, os resultados sugerem que as variantes genéticas desempenham um papel importante na variabilidade da resposta individual da função do ventrículo esquerdo e da tolerância ao exercício físico avaliada antes e após um programa de treino.

Abstract

The aim of the present thesis was to examine the effects of exercise training, gene variants and the interaction between both on left ventricular function, left ventricular structure and exercise tolerance in heart failure patients. Heart failure patients with preserved, mild and moderate to severe reduction of left ventricular ejection fraction (LVEF) were randomly assigned to exercise training plus usual care or usual care alone in a planned randomization ratio of 2:1. Exercise training encompassed 3 sessions per week of aerobic exercise for 6 months. Left ventricular function, left ventricular dimensions, and exercise tolerance were assessed before and after each intervention. Variants located in *ADRB1* Arg389Gly (rs1801253), *GNAS* -1211 G/A (rs6123837), *GNAS* 2291 C/T (rs6026584), *PPARGC1A* Gly482Ser (rs8192678), *PPARGC1A* 2962 G/A (rs6821591), *PPARA* Leu162Val (rs1800206), *PPARA* Intron 7 C/G (rs4253778), *PPARD* 294 C/T (rs2016520) and *NRF2* A/C (rs12594956) genes were assessed with restriction fragment length polymorphism analysis. Exercise training improved significantly left ventricular function and exercise tolerance in all heart failure subsets. Exercise training reduced significantly left ventricular dimensions in patients with moderate to severe reduction of LVEF, but not consistently in patients with mildly reduced and preserved LVEF. Deceleration time of early mitral flow was higher at baseline in *GNAS* -1211G allele carriers compared with -1211A allele homozygotes ($P<0.05$). Exercise training attenuated deceleration time in -1211G allele carriers ($P<0.05$) but not in -1211A allele homozygotes. Similarly, *PPARGC1A* Ser482 allele homozygotes had lower LVEF at baseline compared with heterozygotes ($P=0.027$), and values also tended to be lower than Gly482 allele homozygotes ($P=0.059$). Exercise training increased LVEF more pronouncedly in *PPARGC1A* Ser482 allele homozygotes compared with heterozygotes ($P=0.037$), but not when compared with Gly482 allele homozygotes ($P=0.170$). *ADRB1* 389Gly homozygotes had a greater training-induced increase in exercise tolerance than 389Arg homozygotes ($P=0.04$). In addition, the exercise training-induced increase in exercise tolerance was more pronounced in *PPARA* 162Val carriers than in 162Leu homozygotes ($P=0.032$). Furthermore, exercise training increased exercise tolerance in *NRF2* A allele carriers ($P<0.005$), but not in G allele homozygotes. These data indicate that exercise training improves left ventricular function and exercise tolerance in all heart failure subsets. Furthermore, the current observations suggest that gene variants play important roles in the variation of left ventricular function and exercise tolerance that is observed at baseline and in response to exercise training.

List of Abbreviations

ACE: Angiotensin-I converting enzyme

ADRB1: $Beta_1$-adrenergic receptors

ATI: Angiotensin-II type I receptor

BMI: Body mass index

DNA: Deoxyribonucleic acid

DT: Deceleration time of early filling velocity

E/A ratio: Early to late mitral inflow velocity ratio

ECG: Electrocardiogram

EDD: End-diastolic diameter

EDTA: Ethylenediamine tetraacetic acid

ESC: European Society of Cardiology

ESD: End-systolic diameter

GABPA: GA binding protein transcription factor

GNAS: Adenylate cyclase-stimulating G alpha protein

GRK5: G protein-coupled receptor kinase 5

GWAS: Genome-wide association scan

HF-ACTION: Heart Failure: A Controlled Trial Investigation Outcomes of Exercise Training trial

HFPEF: Heart failure with preserved left ventricular ejection fraction

HFREF: Heart failure with reduced left ventricular ejection fraction

HWE: Hardy-Weinberg equilibrium

LRIG3: Leucine-rich repeats and immunoglobulin-like domains 3 gene

LV: Left ventricle

LVEF: Left ventricular ejection fraction

MET: Metabolic equivalent

miRNA: Micro ribonucleic acid

mph: Miles per hour

mRNA: Messenger ribonucleic acid

NRF1: Nuclear respiratory factor 1

NRF2: Nuclear respiratory factor 2

NSTEMI: Non-ST segment elevation myocardial infarction

NYHA: New York Heart Association

PCR: Polymerase chain reaction

PDBP: Peak diastolic blood pressure

PHR: Peak heart rate

PPARA: Peroxisome proliferator-activated receptor alpha

PPARD: Peroxisome proliferator-activated receptor delta

PPARGC1A: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

PSBP: Peak systolic blood pressure

RDBP: Resting diastolic blood pressure

RHR: Resting heart rate

RSBP: Resting systolic blood pressure

SERCA2a: Sarcoplasmic reticulum Ca^{2+} ATPase

SNP: Single nucleotide polymorphism

STEMI: ST segment elevation myocardial infarction

TFAM: Transcription factor A

UTR: Untranslated region

USP3: Ubiquitin-specific protease 3 gene

VO_2max: Maximal oxygen consumption

CHAPTER I

INTRODUCTION

Introduction

Heart failure is a prominent health problem worldwide. In recent years, the incidence of heart failure has continued to increase and its morbidity and mortality remained elevated (Bleumink, et al., 2004; Djousse, Driver, & Gaziano, 2009; Lloyd-Jones, et al., 2002). In the presence of this pathological condition, several neurohormonal and signalling cascades are activated to maintain cardiac function within the normal range (Mann & Bristow, 2005). However, these mechanisms become deleterious after prolonged activation, promoting undesirable changes in cardiac function and structure (Mann & Bristow, 2005). These changes often referred to as cardiac remodelling are accompanied by alterations in genome expression as well as molecular, cellular and interstitial modifications (Cohn, Ferrari, & Sharpe, 2000). It is generally accepted that as heart failure progresses, cardiac remodelling ensues (Gaudron, Eilles, Kugler, & Ertl, 1993; Mitchell, Lamas, Vaughan, & Pfeffer, 1992; Rumberger, Behrenbeck, Breen, Reed, & Gersh, 1993), cardiac function deteriorates and fatigue and exercise intolerance become evident (Cohn, et al., 2000). Thus, it is not surprising that all these elements are important indicators of poor prognosis in heart failure patients (Jorge Alves, et al., 2010).

There is unequivocal evidence to support that exercise training improves exercise tolerance in heart failure patients (Belardinelli, Georgiou, Scocco, Barstow, & Purcaro, 1995; Giannuzzi, Temporelli, Corra, & Tavazzi, 2003; Gielen, et al., 2003; Hambrecht, et al., 2000; Hambrecht, et al., 1995). This is an important benefit given that exercise capacity is an important determinant of health-related quality of life and a strong indicator of prognosis in heart failure patients (Jorge Alves, et al., 2010; Kitzman, 2011). Nonetheless, studies until now have focused their attention on the therapeutic role of exercise training in heart failure patients with reduced left ventricular ejection fraction (HFREF). This issue is of paramount clinical importance as recent estimates indicate that these patients comprise only 50% of the heart failure population (Hogg, Swedberg, & McMurray, 2004). At the same time, recent estimates indicate that almost half of the heart failure population presents preserved left ventricular ejection fraction (HFPEF) (Hogg, et al., 2004; Maeder & Kaye, 2009). Moreover, mounting evidence indicates that exercise tolerance can be as reduced in patients with HFPEF as

is in those with HFREF (Kitzman, et al., 2002). Recent studies have suggested that exercise training may improve exercise tolerance in patients with HFPEF (Gary, et al., 2004; Smart, Haluska, Jeffriess, & Marwick, 2007). However, the mechanisms by which endurance training improves exercise tolerance in these patients remain unclear. Given that these patients are commonly female and older (Hogg, et al., 2004), and given the presence of multiple cardiovascular abnormalities, one simple mechanism is unlikely to explain exercise intolerance (Borlaug & Paulus, 2011; Maeder & Kaye, 2009). The list of potential factors includes impaired left ventricular (LV) diastolic function and blunted lusitropic, chronotropic, vasomotor, and inotropic responses to exercise (Figure 1) (Fontes-Carvalho & Leite-Moreira, 2011; Paulus, 2010). Among these, an increased slope of end-diastolic pressures relative to end-diastolic volumes and blunted diastolic reserve are considered to be the prime suspects of exercise intolerance in HFPEF (Chattopadhyay, et al., 2010; Paulus, 2010). The exertional diastolic dysfunction in combination with blunted preload reserve may prevent adequate increases in cardiac output, contributing to exercise intolerance (Ha, et al., 2005; Little, Kitzman, & Cheng, 2000) Ventricular and vascular stiffening are also elevated in patients with HFPEF (Kawaguchi, Hay, Fetics, & Kass, 2003). Abnormal ventricular-vascular responses during exercise are associated with blunted contractile reserve and weak reductions in arterial afterload, both of which contribute to exercise intolerance (Borlaug, Olson, et al., 2010; Chantler, Lakatta, & Najjar, 2008). In other words, LV arterial elastance (Ea), a measure of vascular stiffness, has been reported to increase more during exercise in patients compared with controls, while LV end-systolic elastance (Ees), a measure of LV contractile function appears to increase less in patients than controls (Phan, et al., 2009). Thus, ventricular vascular coupling (Ea/Ees ratio) remains unchanged or slightly decreases during exercise in patients with HFPEF while it appears to decrease more pronouncedly in healthy individuals (Borlaug, Olson, et al., 2010; Phan, et al., 2009). There is also evidence showing that endothelial dysfunction is present and systemic vascular resistance is increased in patients with HFPEF, abnormalities that are associated with decreased blood flow to skeletal muscle and reduced stroke volume index at peak exercise, respectively (Borlaug, et al., 2006; Borlaug, Olson, et al., 2010; Maeder, Thompson, Brunner-La Rocca, & Kaye, 2010). In some patients, chronotropic incompetence can also be present, which in combination with blunted stroke volume may prevent

increases in cardiac output during exercise (Borlaug, et al., 2006; Brubaker, et al., 2006). Instead of a single factor, these data confirm that the culmination of several abnormalities impairing cardiovascular functional reserve is associated with exercise intolerance in patients with HFPEF (Borlaug, Olson, et al., 2010). This notion has been corroborated in an elegant and comprehensive study, which demonstrated that patients with HFPEF have an increased number of cardiovascular reserve abnormalities compared with hypertensive and control subjects (Borlaug, Olson, et al., 2010). Moreover, the authors of this study (Borlaug, Olson, et al., 2010) showed that the presence of a greater number of reserve abnormalities is associated with more severely depressed exercise tolerance. Besides abnormalities in cardiovascular reserve function, a recent investigation showed that exercise intolerance is not only associated with peak cardiac output but also with peak arteriovenous oxygen content difference (Haykowsky, et al., 2011). Therefore, peripheral factors seem to be also important contributors to exercise intolerance in HFPEF.

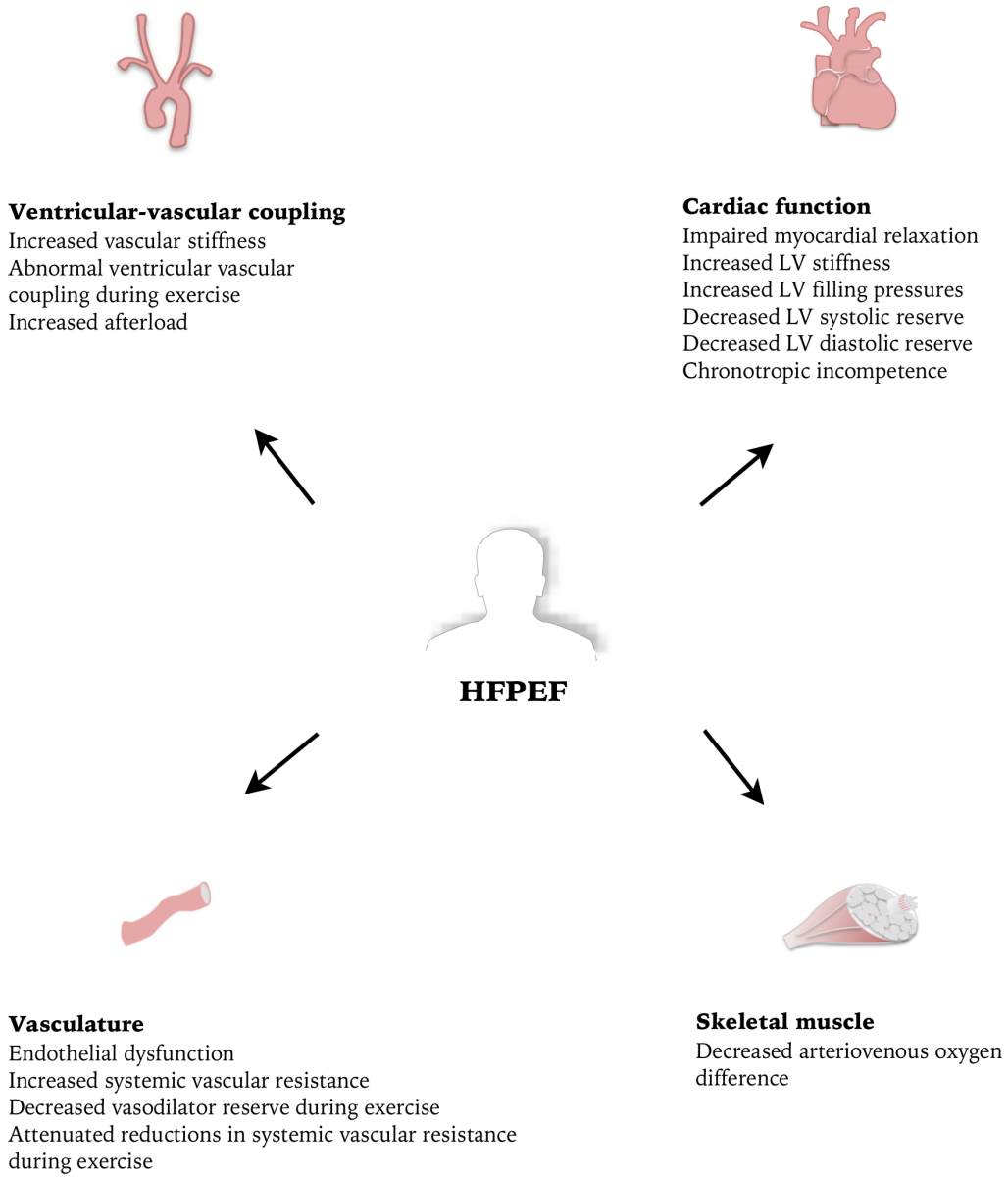

Figure 1. Pathophysiological processes in HFPEF

Once considered a harmful stimulus to the diseased heart, exercise training and its capacity to reverse the pathological remodelling of the failing heart have been increasingly appreciated. Initial studies in patients and animals with myocardial infarction raised concerns regarding the harmful effects of exercise training on cardiac remodelling. Several opposing studies dismissed these observations by demonstrating that endurance training does not harm (Cobb, et al., 1982; Dubach, Myers, Dziekan, Goebbels, Reinhart, Muller, et al., 1997; Dubach, Myers, Dziekan, Goebbels, Reinhart, Vogt, et al., 1997; Giallauria, et al., 2006; Giannuzzi, et al., 1993; Jette, Heller, Landry, & Blumchen, 1991; Otsuka, et al., 2003), and may actually benefit the damaged heart (Giallauria, et al., 2008; Giannuzzi, et al., 1997; Haykowsky, et al., 2007). LV systolic dysfunction and diastolic dysfunction are hallmarks of heart failure, and commonly accompany the signs and symptoms that characterize heart failure patients. The disassociation of left ventricular performance at rest with functional capacity has led several researchers to claim that cardiorespiratory fitness has little to do with the heart in heart failure patients. While it has been demonstrated that changes in cardiorespiratory fitness are related with skeletal muscle oxidative capacity (Gielen, et al., 2005; Hambrecht, et al., 1995), evidence exists indicating that exercise training may improve ventricular function and may provide benefits that go above and beyond those promoted by changes in the oxygen extraction from skeletal muscles (Belardinelli, Georgiou, Cianci, & Purcaro, 1996; Sullivan, Higginbotham, & Cobb, 1988). However, the literature concerning the effects of exercise training presents inconsistent results. For example, several studies have reported that peak, but not resting, cardiac output, stroke volume and LV ejection fraction (LVEF) improve in heart failure patients with systolic dysfunction after 8 weeks of moderate to high intensity exercise training (Belardinelli, et al., 1996; Coats, et al., 1992; Dubach, Myers, Dziekan, Goebbels, Reinhart, Muller, et al., 1997). On the other hand, systolic function at rest has been shown to improve with exercise training programmes that lasted 12 weeks or more and incorporated moderate intensity exercise (Beckers, et al., 2008; Delagardelle, et al., 2008; Giannuzzi, et al., 2003; Klecha, et al., 2007; Wisloff, et al., 2007). The therapeutic role of exercise training on ventricular function is further supported by evidence, in which contractile function did not improve in patients who received only standard medical care (Giannuzzi, et al., 2003; Haykowsky, et al., 2007; Klecha, et al., 2007).

It is important to note that these outcomes were commonly attained on top of pharmacological treatment with established antiremodelling actions, indicating that exercise training activates mechanisms beyond those promoted by pharmacological intervention.

With respect to LV diastolic function, the evidence is very limited and inconclusive. Very few studies have tested the impact of exercise training in heart failure patients, half of which showed no effects on diastolic function. A preliminary study (Belardinelli, et al., 1996) showed that diastolic function at rest and during exercise improves with short-term and moderate intensity exercise training. These findings were not supported in three subsequent studies (Karapolat, et al., 2009; Klocek, Kubinyi, Bacior, & Kawecka-Jaszcz, 2005; Parnell, Holst, & Kaye, 2002), in which the ratio of early mitral (E) and late (A) filling velocities, the deceleration time of early mitral flow and the isovolumic relaxation time remained unchanged after exercise training. In contrast, another investigation using cardiac magnetic resonance imaging showed that exercise training improves left ventricular diastolic relaxation velocity (Myers, et al., 2002). Wisloff *et al.* (2007) also showed that LV filling pressure and isovolumic relaxation time decrease considerably after high intensity exercise training. Given that abnormal left ventricular relaxation and diastolic filling pressure are both associated with exercise intolerance (Barmeyer, Mullerleile, Mortensen, & Meinertz, 2009; Cheng, Noda, Nozawa, & Little, 1993; Little, et al., 2000), it follows that exercise training may improve diastolic function and by this means functional capacity in heart failure patients. However, considering all the studies thus far, the impact of exercise training on diastolic function remains an unresolved issue and more studies are clearly warranted to elucidate this relationship (Zile & Brutsaert, 2002; Zile, et al., 2001).

Although data indicate that exercise training can improve cardiac function in heart failure patients with HFREF (Haykowsky, et al., 2007; van Tol, Huijsmans, Kroon, Schothorst, & Kwakkel, 2006), it remains unclear whether exercise training improves cardiac function and remodelling in patients with HFPEF (Gary, et al., 2004). In the presence of normal systolic function, the cause of exercise intolerance and fatigue in HFPEF has long been considered to be related with diastolic dysfunction (Soufer, et al., 1985). Indeed, diastolic dysfunction plays a central role in the pathophysiology of

HFPEF, as the presence of impaired myocardial relaxation, increased LV chamber stiffness and elevated LV filling pressures has been documented in most patients with this condition (Zile, Baicu, & Gaasch, 2004; Zile, et al., 2001). In these patients, increased ventricular stiffness displaces the entire diastolic pressure–volume relation upward and to the left (Fontes-Carvalho & Leite-Moreira, 2009; Leite-Moreira, 2006). As a result, elevated filling pressures are required for attainment of a given ventricular volume in a patient with increased ventricular stiffness compared with a normal individual (Fontes-Carvalho & Leite-Moreira, 2009). Furthermore, the slope of end-diastolic pressure-volume relation becomes steeper in patients with increased ventricular stiffness (Zile, et al., 2004), which means that small variations in central volume result in large variations in intraventricular pressures and ventricular filling pressures (Ferreira-Martins & Leite-Moreira, 2010; Fontes-Carvalho & Leite-Moreira, 2009). In addition to impaired diastolic function, recent studies have demonstrated that diastolic reserve is also reduced in patients with HFPEF (Chattopadhyay, et al., 2010; Phan, et al., 2009). Under normal conditions, LV diastolic pressures decrease during exercise (Little, et al., 2000). Hence, atrio-ventricular pressure gradient and left ventricular filling rate increase allowing stroke volume to be maintained during elevated heart rates without elevation of left atrial pressures (Little, et al., 2000). In contrast, heart failure patients rely on enhanced left atrial pressures to increase left ventricular filling during exercise, due to slower LV relaxation and increased diastolic pressures (Little, et al., 2000). Increased LV filling pressures may not be accompanied by increases in end-diastolic volumes during exercise, revealing the limited ability that the non-compliant left ventricle may possess to use the Frank-Starling mechanism, a limitation that may prevent patients with diastolic dysfunction from increasing stroke volume during exercise (Kitzman, Higginbotham, Cobb, Sheikh, & Sullivan, 1991; Zile, et al., 2004). Indeed, although LVEF is preserved at rest, systolic function has been shown to be abnormal during exercise in patients with HFPEF (Kitzman, et al., 1991). These observations have been confirmed by recent studies, where relative changes in stroke volume and cardiac output during exercise were lower in patients compared with controls (Borlaug, Nishimura, Sorajja, Lam, & Redfield, 2010; Maeder, et al., 2010; Phan, et al., 2009). Indeed, blunted increases in stroke volume, cardiac output and cardiac contractility during exercise have been associated with exercise intolerance in patients with HFPEF (Borlaug, Olson, et al., 2010; Maeder, et al.,

2010). Furthermore, a number of independent studies have reported reduced systolic and diastolic mitral annular amplitudes and velocities in patients compared with controls (Brucks, et al., 2005; Phan, et al., 2009; Yip, et al., 2002). In addition, a recent investigation using speckle-tracking echocardiography showed that both systolic longitudinal and radial strain are blunted at rest and fail to rise normally during exercise in patients with HFPEF (Tan, et al., 2009). Together, these data indicate that subtle abnormalities in cardiac function at rest become notorious during exercise in patients with HFPEF. However, the impact of exercise training in cardiac function and remodelling in these patients is yet to be determined. Considering all the present data, the present work aims to examine the effects of exercise training on the cardiac function and structure as well as exercise tolerance in heart failure patients with preserved, mild and moderate to severe systolic dysfunction (expressed as reduced LVEF).

The portfolio of proteins that contribute to cardiac remodelling and heart failure is immense and encompasses cell membrane receptors (El-Armouche & Eschenhagen, 2009; Mudd & Kass, 2008), calcium cycling regulatory proteins (El-Armouche & Eschenhagen, 2009; Lehnart, Maier, & Hasenfuss, 2009), cytoskeletal and sarcomeric proteins (Hamdani, et al., 2008; Hein, Kostin, Heling, Maeno, & Schaper, 2000), pro-inflammatory cytokines (Feldman, et al., 2000; Kleinbongard, Heusch, & Schulz, 2010) as well as a host of protein kinases and phosphatases that modulate cellular behaviour (Ikeda, Hoshijima, & Chien, 2008). The expression and activities of proteolytic enzymes that are responsible for remodelling the extracellular matrix, known as matrix metalloproteinases, are also altered in human heart failure in tandem with ventricular remodelling (Spinale, 2007; Spinale, et al., 2000). Heart failure is also associated with a wide range of metabolic abnormalities, including decreases in fatty acid oxidation and glucose metabolism, disturbed oxidative phosphorylation and impaired energy transfer (Ingwall, 2009; Neubauer, 2007).

The molecular portrayal is, however, incomplete and molecular and genetic studies continue to recognize genes and signalling cascades that participate in the development and progression of heart failure (Liew & Dzau, 2004). In addition to disease-causing (rare) mutations (Liew & Dzau, 2004), investigators have striven to identify common genetic variants (single nucleotide polymorphisms) that might be

associated with an increased risk of developing heart failure (Alves, Eynon, Oliveira, & Goldhammer, 2010; Raynolds, et al., 1993; Small, Wagoner, Levin, Kardia, & Liggett, 2002). Most studies so far have found no differences in the prevalence of disease predisposing alleles among heart failure patients and controls (Alves, et al., 2010). However, the genetic predisposition to heart failure has been suggested in studies using different analytical approaches. For example, it has been reported that the incidence rates of heart failure are almost twice as high in offspring with a parent with heart failure as in those without a parent with heart failure (Lee, et al., 2006). Moreover, individuals with at least one parent with heart failure are more likely to have increased LV mass, dimensions and systolic dysfunction compared with those who do not have a parent with heart failure (Lee, et al., 2006).

Common variants in genes that encode neurohormonal, adrenergic, intracellular, interstitial and vascular proteins have also been reported to modulate the responses to pharmacological treatment and its effect on hospital admissions and survival (Cresci, et al., 2009; Mizon-Gerard, et al., 2004; Raynolds, et al., 1993). For example, common variants in genes encoding proteins of the sympathetic nervous system (Biolo, et al., 2008; Borjesson, Magnusson, Hjalmarson, & Andersson, 2000; Liggett, et al., 2006; Liggett, et al., 1998) and renin-angiotensin-aldosterone system (McNamara, et al., 2001; McNamara, et al., 2004; McNamara, et al., 2006) were associated with an increased risk of death. As neurohormonal activation is implicated in cardiac dysfunction and remodelling (Bristow, et al., 1982), it may be argued that gain-of-function variants may accelerate the evolution of heart failure (Mialet Perez, et al., 2003). On the other hand, the adverse clinical outcomes associated with these 'unfavourable' genetic variants often improved with pharmacological treatment (Biolo, et al., 2008; Cresci, et al., 2009; Liggett, et al., 2006; Liggett, et al., 1998; McNamara, et al., 2001; McNamara, et al., 2004; McNamara, et al., 2006). It is worth mentioning that other studies have failed to find such associations between genetic variants and clinical end-points, including all cause hospital admissions and survival (Sehnert, et al., 2008; White, et al., 2003). Even though it remains unclear which and how gene variants modulate the outcomes of heart failure, there is evidence to suggest that the strongest effect might come from multiple variants interacting with each other (gene-

gene interaction) (de Groote, et al., 2005; Shin, et al., 2007) and with the environment (gene-environment interaction) (Cresci, et al., 2009; Shin, et al., 2007).

The evolution of cardiac dysfunction and remodelling has also been associated with variants that are located in genes with important roles in the heart failure process (Andersson & Sylven, 1996; Barbato, et al., 2007; Biolo, et al., 2008; Chen, et al., 2007; McNamara, et al., 2006; Sanderson, et al., 1999; Terra, et al., 2005; Tiago, et al., 2002). One of these variants has been identified in the gene that encodes the β1-adrenergic receptor (ADRB1) (Mason, Moore, Green, & Liggett, 1999). The function of this receptor is crucial for the regulation of cardiac function but its overactivation has been implicated in cardiac remodelling and heart failure (Triposkiadis, et al., 2009). In the *ADRB1* gene, the presence of an arginine rather than a glycine at codon 389 (Arg389Gly) is associated with increased basal and agonist-stimulated adenylate cyclase activities and with increased cardiac contractility (Mason, et al., 1999). On the other hand, the Arg389 allele variant is also associated with increased receptor desensitization when exposed to excessive concentrations of catecholamines (Mason, et al., 1999; Mialet Perez, et al., 2003). Recent studies have shown that heart failure patients homozygous for the Arg389 allele have greater improvements in cardiac function and greater reductions in cardiac diameters following pharmacological treatment compared with patients harbouring the Gly389 allele (Chen, et al., 2007; Terra, et al., 2005). In line with these observations, variants located in the promoter region and in intron 1 of the stimulatory Gα protein (GNAS) gene have been associated with increased basal and agonist-stimulated adenylate cyclase activities, as well as with increased cardiac contractility in patients with cardiovascular disease (Frey, et al., 2009; Frey, et al., 2008). However, whether these variants influence cardiac function and remodelling in heart failure patients is yet to be determined.

The association of genetic variants with cardiac and exercise tolerance responses to exercise training has also been reported in a small number of studies conducted in healthy subjects (Danser, et al., 1995; Montgomery, et al., 1998). Amidst these genetic variants, some have been located in genes involved in the regulation of cardiac and skeletal muscle metabolism (Jamshidi, et al., 2002; Stefan, et al., 2007). The peroxisome proliferator-activated receptor γ coactivator-1α (PPARGC1A) controls cardiac metabolism and mitochondrial respiration through the coordinate coactivation

of peroxisome proliferator–activated receptors (PPARs) and nuclear respiratory factors 1 (NRF1) and 2 (NRF2), which regulate the transcription of genes that encode proteins involved in fatty acid oxidation, mitochondrial biogenesis and oxidative phosphorylation (Finck & Kelly, 2006; Ventura-Clapier, Garnier, Veksler, & Joubert, 2011). The expression of PPARGC1A has been shown to increase with exercise training in cardiac and skeletal muscle (Kemi, et al., 2007; Pilegaard, Saltin, & Neufer, 2003; Wisloff, et al., 2007) and to decrease in heart failure (Garnier, et al., 2003; Ingwall, 2009; Kemi, et al., 2007). Consistent with these observations, fatty acid oxidation and mitochondrial function have also been demonstrated to increase with exercise training (Baar, et al., 2002; Holloszy, Kohrt, & Hansen, 1998; Pilegaard, et al., 2005; Terada, Kawanaka, Goto, Shimokawa, & Tabata, 2005) and to decrease in heart failure (Arany, et al., 2005; Sack, et al., 1996; Sihag, Cresci, Li, Sucharov, & Lehman, 2009). Increased expression of PPARGC1A has also been reported in human heart failure, perhaps representing a mechanism activated to compensate for the reduced mitochondrial DNA content (Karamanlidis, et al., 2010; Sihag, et al., 2009). These data indicate that PPARGC1A regulates cardiac metabolism and mitochondrial function under normal and pathological conditions (Ventura-Clapier, Mettauer, & Bigard, 2007). This notion has been supported in several gene-association studies. For example, variants located in the human *PPARGC1A* gene (Ek, et al., 2001; Stefan, et al., 2007) have been associated with endurance performance in response to exercise training (He, et al., 2008), but also with metabolic and cardiovascular diseases (Ek, et al., 2001; Ingelsson, et al., 2008; Wang, et al., 2007). These observations suggest that certain variants can be associated with both favourable and unfavourable responses, perhaps depending on the environmental conditions. Moreover, variants located in the *PPARA* and *NRF2* genes have been associated with endurance performance and exercise tolerance responses to exercise training (Eynon, Alves, Sagiv, Yamin, & Meckel, 2010; Eynon, Meckel, et al., 2010; He, et al., 2007). In addition, skeletal muscle fibre composition and cardiac responses to exercise training have been associated with an intronic variant located in the human *PPARA* gene (Ahmetov, et al., 2006; Jamshidi, et al., 2002). PPARA is a ligand-activated transcription factor that regulates the expression of genes involved in fatty acid uptake and oxidation, lipid metabolism, and inflammation (Fruchart, Duriez, & Staels, 1999; Jamshidi, et al., 2002). NRF1 and NRF2 have a central role in mitochondrial biogenesis, controlling

the transcription of genes encoding mitochondrial proteins (Kelly & Scarpulla, 2004) and promoting the transcription of transcription factor A (TFAM), a nuclear-encoded transcription factor essential for replication and transcription of mitochondrial DNA (Gleyzer, Vercauteren, & Scarpulla, 2005). The expression of PPARA and NRF2 has been shown to decrease in heart failure in tandem with reduced fatty acid oxidation and impaired mitochondrial function (Garnier, et al., 2003; Lopaschuk, Ussher, Folmes, Jaswal, & Stanley, 2010; Stanley, Recchia, & Lopaschuk, 2005; Ventura-Clapier, et al., 2011). In contrast, exercise training increases fatty acid oxidation, improves mitochondrial respiration in cardiac and skeletal muscle, and increases the expressions of PPARA and NRF2 (Terada, et al., 2005; Terada, et al., 2001). Thus, these data indicate that PPARA and NRF2 also regulate cardiac metabolism as well as mitochondrial function under normal and pathological conditions.

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CHAPTER II

STRUCTURE AND AIMS OF THE THESIS

Structure

The present thesis was organized into five chapters. The introduction to the literature supporting the aims of the present thesis was provided in **chapter I**. The main aim of the thesis and the purposes of each study are outlined in **chapter II**. The experimental work is presented in **chapter III**. The experimental work is divided into four original studies, each of which attempts to address specific research questions. The methodological and overall discussions are presented in **chapter IV**. The main conclusions of the present thesis are outlined in **chapter V**.

Aims

The main aim of the present thesis was to examine the effects of exercise training and genetic variants on left ventricular function, left ventricular structure and exercise tolerance in heart failure patients. The effect of genotype x exercise training interaction on left ventricular function and exercise tolerance in heart failure patients was also analysed.

Title, aims and status of each study

Study I

Title: Exercise training improves exercise tolerance and cardiac function in heart failure patients with preserved ejection fraction

Authors: Alberto J Alves, Ehud Goldhammer, Fernando Ribeiro, José A Duarte, Yelena Rivlin, Uri Rosenschein, João L Viana, Michael Sagiv, José Oliveira.

Aim: To examine the effect of exercise training on the exercise tolerance, left ventricular function and exercise hemodynamics in heart failure patients with preserved left ventricular ejection fraction

Status: Submitted

Study II

Title: Exercise Training Improves Diastolic Function in Heart Failure Patients

Authors: Alberto J Alves, Fernando Ribeiro, Ehud Goldhammer, Yelena Rivlin, Uri Rosenschein, João L Viana, José A Duarte, Michael Sagiv, José Oliveira.

Aim: To analyze the effects of exercise training on exercise tolerance, left ventricular function and structure in heart failure patients with preserved, mild and moderate to severe reduction of left ventricular ejection fraction

Status: Published ahead of print in *Medicine & Science in Sports & Exercise*. DOI: 10.1249/MSS.0b013e31823cd16a

Study III

Title: *GNAS* A-1121G variant is associated with improved diastolic dysfunction in response to exercise training in heart failure patients.

Authors: Alberto J Alves, Ehud Goldhammer, Fernando Ribeiro, Nir Eynon, Sigal Ben-Zaken Cohen, José A Duarte, João L Viana, Michael Sagiv, José Oliveira.

Aim: To investigate whether *ADRB1* Arg389Gly (rs1801253), *GNAS* -1211 G/A (rs6123837) and *GNAS* 2291 C/T (rs6026584) gene variants are associated with left ventricular function and exercise tolerance at baseline or in response to exercise training in heart failure patients.

Status: Submitted

Study IV

Title: Genetic variation in the PPARGC1A signalling cascade modulates cardiac function and exercise tolerance responses to exercise training in heart failure patients

Authors: Alberto J Alves, Ehud Goldhammer, Alejandro Lucia, Nir Eynon, Michael Sagiv, João L Viana, Fernando Ribeiro, José A Duarte, José Oliveira.

Aim: To investigate whether variants in the *PPARGC1A* (rs8192678, rs6821591), *PPARA* (rs1800206, rs4253778), *PPARD* (rs2016520) and *NRF2* (rs12594956) genes are associated with left ventricular function and exercise tolerance at baseline or in response to exercise training in heart failure patients.

Status: Submitted

CHAPTER III

EXPERIMENTAL WORK

Study I

Exercise training improves exercise tolerance and cardiac function in heart failure patients with preserved ejection fraction

Alberto J Alves, Ehud Goldhammer [2,3], Fernando Ribeiro[1,6], José A Duarte [1], Yelena Rivlin [2,3], Uri Rosenschein [2,3], João L Viana [4], Michael Sagiv [5], José Oliveira [1],

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Submitted

Abstract

Aims: To examine the effect of exercise training on exercise tolerance, left ventricular function and exercise hemodynamics in heart failure patients with preserved ejection fraction (HFPEF)

Methods and results: Fifty-one patients were randomly assigned to exercise training plus usual care (n=33) or usual care alone (n=18) in a planned randomization ratio of 2:1. Left ventricular ejection fraction (LVEF), pulsed-wave Doppler mitral inflow velocities, left ventricular dimensions, exercise performance (METs), and hemodynamic response to exercise were assessed before and after completing each intervention. Exercise tolerance increased with exercise training (P=0.002), while it was unchanged with usual care alone. Exercise training increased significantly LVEF (P<0.001) and mean ratio of early to late mitral inflow velocity (E/A ratio, P<0.001), and decreased deceleration time of early mitral inflow (DT, P<0.001), whereas these variables did not change with usual care alone. In contrast, left ventricular dimensions did not change with both interventions. Exercise training decreased resting heart rate and increased heart rate reserve, but these changes became non-significant after correction for multiple comparisons. No more differences were observed in the hemodynamic response to exercise between exercise training and with usual care alone groups.

Conclusions: This study indicates that exercise training improves exercise tolerance and left ventricular function in patients with HFPEF, suggesting that exercise training is an effective non-pharmacological treatment to improve clinical status of heart failure patients.

Introduction

The traditional understanding of heart failure has changed in recent years, with evidence indicating that almost one half of patients with heart failure have preserved left ventricular ejection fraction (HFPEF) [1]. Compared with heart failure patients with reduced left ventricular ejection fraction (HFREF), HFPEF patients are older, more often female and have a high prevalence of hypertension, obesity and diabetes as well as several comorbidities such as atrial fibrillation, anaemia and renal failure [1, 2]. Furthermore, the prevalence and prognosis of HFPEF is worsening [3], and recent studies have shown that its clinical presentation can be as serious as that in patients with HFREF [4].

Diastolic dysfunction plays a central role in the pathophysiology of HFPEF, as most patients present impaired myocardial relaxation and increased left ventricular stiffness [5]. Mitral inflow velocities and deceleration time have also been shown to be abnormal in more than half of patients with HFPEF [6]. Furthermore, there is evidence showing that mitral inflow patterns are associated with the severity and prognosis of heart failure, at least in patients with HFREF [7, 8]. Thus, worsening diastolic dysfunction appears to play an important role in the appearance of symptoms and progression of HFPEF [9].

Exercise intolerance is the cardinal symptom in patients with heart failure, and an important determinant of health status [10, 11]. Numerous investigations have shown that exercise tolerance is also severely reduced in patients with HFPEF [12, 13]. Because these patients are usually advanced in age with multiple comorbidities, exercise intolerance should be a central goal in the treatment of patients with HFPEF [14, 15]. Nonetheless, the mechanisms of exercise intolerance in HFPEF remain unclear. It has been suggested that diastolic dysfunction is an important contributor to exercise intolerance in patients with HFPEF [4, 16]. Moreover, growing evidence indicates that blunted left ventricular systolic reserve and chronotropic incompetence could also contribute to exercise intolerance in patients with HFPEF [17, 18].

It has been widely demonstrated that exercise training can improve exercise tolerance, left ventricular function and hemodynamic response in patients with HFREF [19, 20]. Two recent studies have demonstrated that exercise training could improve exercise tolerance in patients with near normal and normal ejection fraction [21, 22], but little

is known concerning its impact on left ventricular function and exercise hemodynamics. Therefore, the purpose of this study was to examine the effects of exercise training on exercise tolerance, left ventricular diastolic function and exercise hemodynamics in patients with HFPEF.

Methods

Patient recruitment

Fifty-one heart failure patients in NYHA (New York Heart Association) class I – III with preserved left ventricular ejection fraction (LVEF ≥ 50%) were included in the study. Exclusion criteria included uncontrolled hypertension, unstable angina pectoris, uncontrolled cardiac arrhythmias, significant ischemic electrocardiogram ST-T changes during initial stages of exercise tolerance test (Modified Bruce protocol), uncontrolled metabolic disease (e.g. uncontrolled diabetes and thyroid disease) and medical conditions that limit the participation in exercise (e.g. peripheral arterial occlusive disease and musculoskeletal disorder). Bnai Zion Medical Center (Israel) ethics committee approved the study protocol and written informed consent was obtained from every patient.

Study design

Patients were randomly assigned to exercise training plus usual care (n=33) or usual care alone (control group, n=18) in a planned randomization ratio of 2:1. Twice as many patients were assigned to the exercise-training group to compensate for the attrition and lower adherence in patients with heart failure who participate in cardiac rehabilitation programs [23]. All patients who completed more than 80% of prescribed exercise sessions were included in the analysis. Usual care consisted of regular appointments with a cardiologist and optimized medication. Patients in the control group did not receive instructions or any form of exercise training. Patients in both groups were assessed at baseline and 6 months after randomization.

Exercise Training

Patients trained three times a week for 6 months. In the first month of training, each exercise session consisted of 10 min warm-up exercises, 15 min of aerobic exercise and 10 min of cool-down with stretching exercises. The aerobic exercise consisted of

interval training composed by 5 sets of 3-min exercise on a treadmill or bicycle ergometer at 70-75% of the maximal heart rate interspersed with 1-min exercise at 40-50% of maximal heart rate. In the following 5 months, aerobic exercise duration increased progressively up to 35 min by increasing the number and duration of exercise sets (7 x 5 min), while maintaining the duration of active recovery. Exercise intensity was adjusted progressively throughout the study to ensure that all exercise-training sessions were performed within the established heart rate.

Exercise Testing

Each subject performed a symptom limited, graded exercise treadmill test according to a standard Modified Bruce protocol. To examine exercise tolerance, we recorded treadmill exercise time and estimated metabolic equivalents (METs) according to the guidelines provided by the American College of Sports Medicine [24]:

MET = [(speed x 0.1) + (grade x speed x 1.8) + 3.5] / 3.5, where fractional grade is expressed in decimal form and speed in meters per minute (1 mile per hour is equal to 26.8 meters per minute).

Blood pressure (by sphygmomanometer) was assessed at rest and at peak exercise, while heart rate (12-lead electrocardiogram) was recorded at rest, throughout exercise and at regular intervals during recovery until heart rate and blood pressure had returned to baseline. Each subject was monitored throughout the test for ECG and heart rate by the Quinton 4500 stress system. Treadmill tests were terminated according to the guidelines provided by the American College of Sports Medicine [24]. All exercise tests were supervised by the same cardiologist, who was blinded to the treatment assignment.

Echocardiographic evaluation

All subjects underwent a complete resting echocardiography examination using a Siemens-Accuson Sequoia machine (USA). Three consecutive cardiac cycles were analyzed and averaged for each patient by an experienced cardiologist. The LVEF was measured with the modified biplane Simpson's method from the apical 4- and 2-chamber views, whereas left ventricular end-diastolic diameter (EDD) and left ventricular end-systolic diameter (ESD) were measured at M-mode in the parasternal long-axis view. Transmitral inflow velocities were assessed by pulsed-wave Doppler,

with the sample volume placed between the mitral leaflet tips in the apical 4-chamber view. The peak early (E) and late (A) transmitral velocities and the deceleration time (DT) of early filling velocity were assessed and the E/A ratio was calculated for the evaluation of diastolic function.

Statistical analysis

Data normal distribution was confirmed by the D'Agostino & Pearson omnibus test and Shapiro-Wilk normality test. Data were presented as mean ± SD. Continuous variables with non-normal distribution were log-transformed before statistical testing and means were back transformed for presentation. To examine the effect of treatment on exercise tolerance, cardiac function and cardiac dimensions, a two-way mixed-model ANOVA was used to compare results between treatment groups over time (treatment x time). The association between treatments and the proportion of patients who improved exercise tolerance above the clinical threshold (10%) was examined through contingency tables with chi-square statistic. To avoid the inflation of type I error that is associated with multiple comparisons, statistical significance was corrected with Holm-Bonferroni method where appropriate. The chi-squared (χ^2) test was used to examine the prevalence of cardiovascular risk factors and medications in the exercise training and control groups. Mean differences were adjusted for potential confounders (age, gender, functional class, cardiovascular risk factors and medication) where appropriate. $P < 0.05$ was considered indicative of statistical significance.

The sample size was estimated based on the only study that tested the effects of exercise training in patients with HFPEF as well as in the clinical threshold of 10% improvement in exercise tolerance [22]. This analysis revealed that 27 subjects in exercise group and 13 patients in the control group, according to the planned randomization ratio of 2:1, would be required to detect with approximately 80% of probability a difference of 15 % in exercise tolerance at a α-level of 0.05. The total number of patients was increased to take into consideration the number of patients who would be unable to complete the programme or to attend the follow-up evaluations [23].

Results

Fifty-one eligible patients consented to participate in the present study, among whom 1 patient in the exercise-training group (3%) and 2 patients in the control group (11%) were unavailable for follow-up evaluations. None of these patients was excluded due to cardiac reasons.

Patient characteristics

Patient characteristics are described in Table 1. Although not significant, patients from the exercise-training group tended to be older. There were no differences between patients from exercise-training and control groups with respect to gender, body mass index (BMI), NYHA classification, cardiovascular risk factors and medication regime.

Table 1. Demographic and clinical characteristics of patients at baseline in exercise training and control groups

	Exercise (*N*=32)	Control (*N*=16)	P
Age (yrs)	64.5 ± 10.9	58.3 ± 8.5	0.05
Sex (M/F)	21/11	13/3	0.26
BMI (kg/m^2)	28.8 ± 4.9	29.1 ± 5.5	0.87
LVEF (%)	54.4 ± 3.5	54.2 ± 3.1	0.84
NYHA			
I	2 (0.06)	3 (0.19)	0.17
II	16 (0.48)	4 (0.25)	
III	15 (0.45)	9 (0.56)	
IV	0 (0.00)	0 (0.00)	
IHD	16 (0.50)	6 (0.38)	0.24
Valvular disease	4 (0.12)	2 (0.13)	0.16
Hypertension	25 (0.78)	9 (0.56)	0.18
Diabetes Mellitus	13 (0.40)	2 (0.13)	0.09
Smoking habits	3 (0.09)	1 (0.07)	0.31
Beta-blockers	24 (0.75)	14 (0.93)	0.24
Alpha-adrenergic antagonists	1 (0.03)	3 (0.15)	0.09
ACE inhibitors	8 (0.25)	6 (0.40)	0.32
AT1R antagonists	6 (0.19)	4 (0.27)	0.70
Calcium channel blockers	11 (0.34)	2 (0.14)	0.29
Diuretics	4 (0.13)	1 (0.07)	0.33
Statins	26 (0.81)	12 (0.80)	0.54

ACE, Angiotensin-converting enzyme; AT1, Angiotensin-II type 1 receptors; BMI, body mass index; LVEF, Left ventricular ejection fraction; NYHA, New York Heart Association. Data are presented as mean ± SD or absolute frequency (relative frequency).

Left ventricular systolic and diastolic function

Baseline and 6-month follow-up values of left ventricular function in exercise training and usual care alone groups are shown in Table 2. Transmitral inflow velocities could not be measured in two exercise patients who presented atrial fibrillation. LVEF increased significantly with exercise training and remained unchanged in the control group (Figure 1). No difference was found between exercise training and usual care alone groups for the E/A ratio at baseline (Table 2). Exercise training increased significantly the E/A ratio, which remained unchanged in the control group (Figure 2). Because it presented a non-normal distribution in the control group, deceleration time of early mitral flow (DT) was log-transformed in both groups for statistical analysis and back- transformed to the original units for presentation. DT was elevated in the exercise-training group compared with the usual care alone group before the interventions (t = 2.41, P=0.027), but no differences were found after the interventions (t = 1.52, P=0.148). Exercise training decreased significantly DT, while there was no change in the control group (Figure 2). All these variables remained significantly different with exercise training after adjustment for age, gender, cardiovascular risk factors and medication regime as well as after correction with Holm-Bonferroni procedure for multiple comparisons.

Table 2. Baseline and follow-up values of left ventricular function and exercise tolerance in exercise training and control groups

Outcome Measure	Exercise Training Group		Control Group		P (treatment x time)
	Baseline	Follow-up	Baseline	Follow-up	
LVEF (%)	54.4 ± 3.5	56.8 ± 2.9*	54.2 ± 3.1	54.3 ± 2.9	0.004
E/A ratio	0.91 ± 0.12	1.03 ± 0.16‡	0.96 ± 0.15	1.00 ± 0.11	0.006
DT (ms) *	239.1 ± 7.81†	212.6 ± 20.5‡	225.6 ± 5.75	211.9 ± 17.4	0.001
METs	4.0 ± 1.2	4.5 ± 1.4*	4.5 ± 1.5	4.3 ± 1.3	0.002

E/A ratio, ratio between early and late mitral flows; DT, deceleration time of early mitral flow; LVEF, Left ventricular ejection fraction; METs, Metabolic equivalents. * Back-transformed data of deceleration time. † Significantly higher than control group, P=0.027. ‡ Significantly lower than baseline, P<0.0001. * Significantly higher than baseline, P<0.0001.

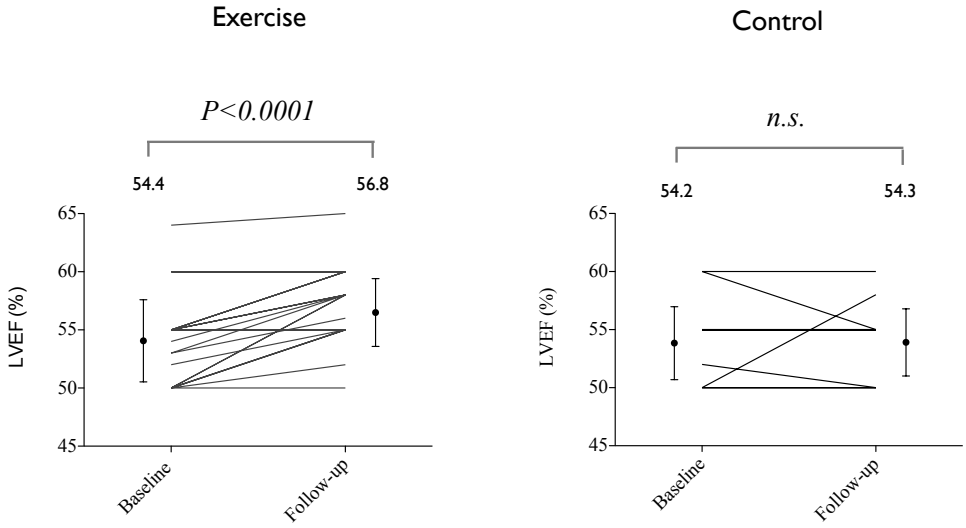

Figure 1. Baseline and follow-up values of left ventricular ejection fraction (LVEF) in exercise training and control groups. The numbers under the bar of significance correspond to the mean values of LVEF (%) before and after the intervention.

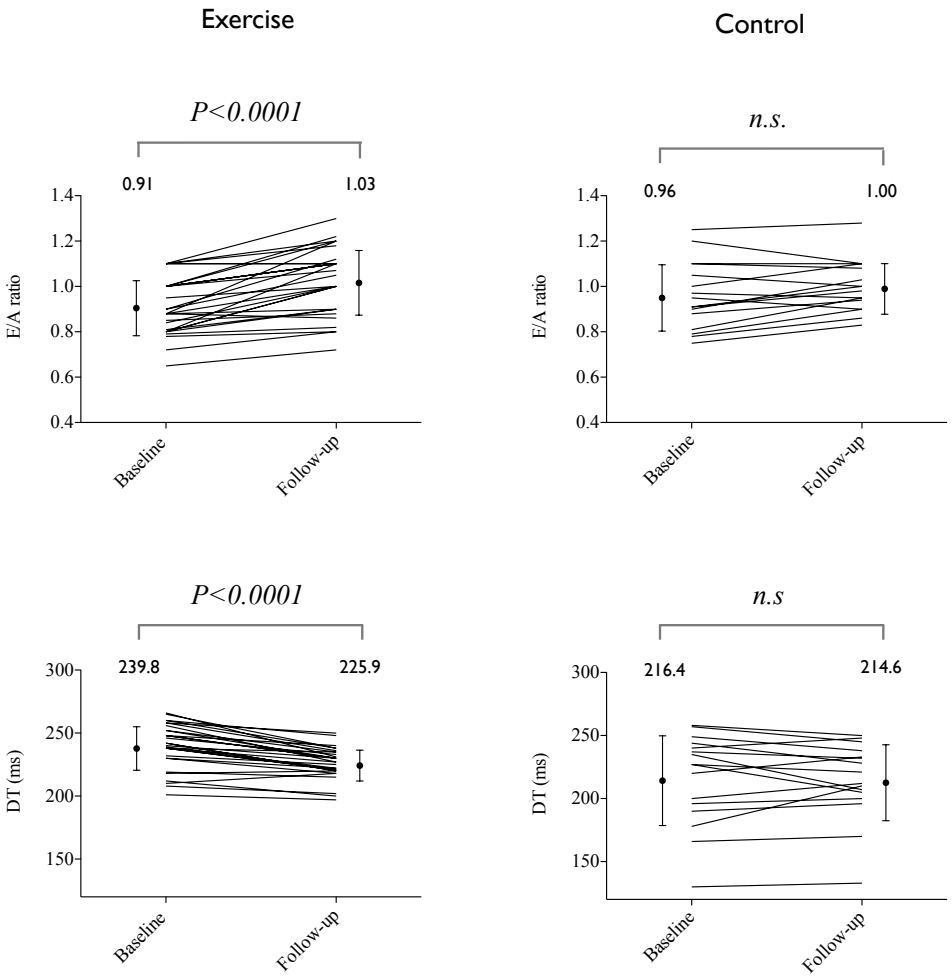

Figure 2. Baseline and follow-up values of E/A ratio and deceleration time of early mitral flow (DT) in exercise training and control groups. In the figure, it is presented the raw (non-transformed) data of deceleration time of early mitral flow (DT).

Left ventricular dimensions

Baseline and follow-up left ventricular diameters in exercise training and control groups are shown in Figure 3. There were no differences between exercise training and control groups in left ventricular dimensions after 6 months of exercise training or usual care alone relative to baseline.

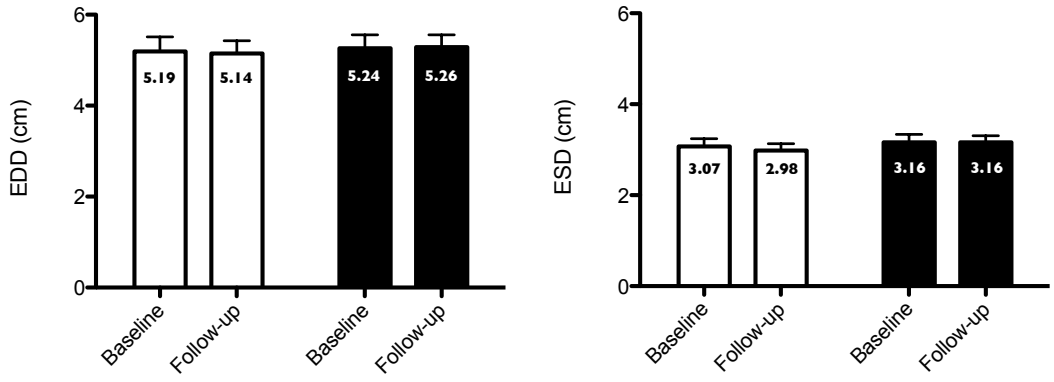

Figure 3. Baseline and follow-up left ventricular dimensions in exercise-training and control groups. The white bars correspond to the exercise-training group, whereas the black bars represent the control group. EDD, end-diastolic diameter; ESD, end-systolic diameter. Data are presented as mean±standard deviation.

Hemodynamic

One patient (3%) in the exercise-training group has not completed the second exercise test and was not included in this analysis. Hemodynamic outcomes in exercise training and usual care groups are shown in Table 3. There were no changes in resting and peak systolic blood pressure and diastolic blood pressure as well as peak heart rate from baseline to follow-up in either group. On the other hand, exercise training decreased resting heart rate and increased heart rate reserve. These variables were non-significant when adjusted with Holm-Bonferroni procedure for multiple comparisons, however.

Table 3. Hemodynamic outcomes in exercise training and control groups

Outcome Measure	Exercise Training Group		Control Group		P (treatment x time)
	Baseline	Follow-up	Baseline	Follow-up	
rHR (bpm)	72.7±14.6	69.2±10.4	69.3±14.9	73.4±9.8	0.03
pHR (bpm)	123.9±21.0	126.4±14.6	129.1±19.0	129.9±14.5	0.58
HRr (bpm)	51.3±21.9	57.2±17.4	59.9±18.4	56.5±11.3	0.01
rSBP (mmHg)	122.9±10.7	127.0±12.8	122.3±12.8	125.9±12.4	0.83
pSBP (mmHg)	156.3 ±14.7	157.5±12.6	160.2±14.5	161.1±15.9	0.94
rDBP (mmHg)	77.7±5.1	76.4±4.7	77.5±6.1	77.3±5.2	0.48
pDBP (mmHg)	77.3±4.8	78.3±5.2	80.0±5.5	81.4±5.4	0.72

rHR, resting heart rate; rSBP, resting systolic blood pressure; rDBP, resting diastolic blood pressure; pHR, peak heart rate; pSBP, peak systolic blood pressure; pDBP, peak diastolic blood pressure; HRr, heart rate reserve.

Exercise tolerance

Exercise training increased significantly exercise tolerance, whereas controls showed a small non-significant reduction in exercise tolerance from baseline (Figure 4). The number of patients with a 10% improvement in exercise tolerance (expressed in METs), which is conventionally used as clinically relevant, was 13 (42%) in the exercise-training group and 0 (0%) in the control group ($\chi^2 = 10.3$, $P = 0.001$).

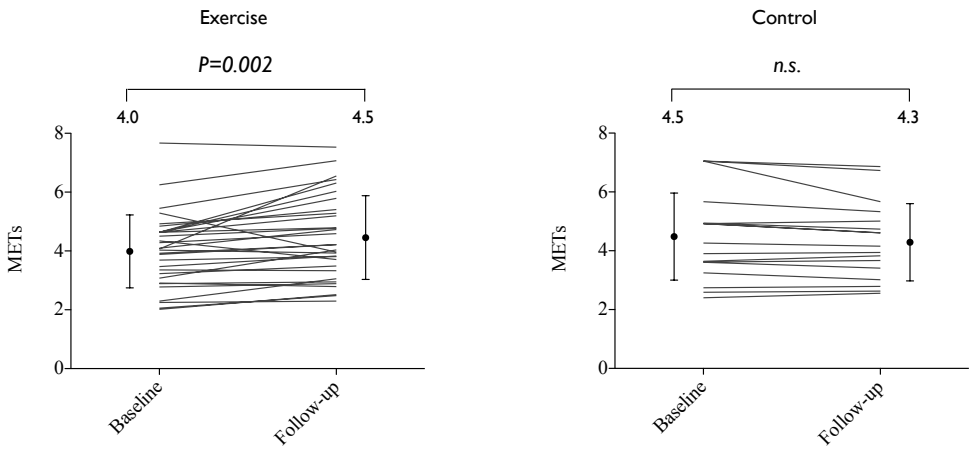

Figure 4. Baseline and follow-up values of exercise tolerance in exercise-training and control groups.

Discussion

Our main findings were that exercise training increased exercise tolerance and improved diastolic dysfunction in patients with HFPEF.

The present study showed that exercise training could improve diastolic function, in particular mitral inflow velocities and deceleration time, in patients with HFPEF. These observations are in agreement with those reported by Edelmann *et al.* [25], which showed that endurance combined with resistance training improves diastolic dysfunction in patients with HFPEF. Thus, these data indicate that moderate intensity endurance training alone or in combination with resistance training is effective in improving diastolic dysfunction in patients with this condition. On the other hand, previous studies have reported that endurance training does not appear to improve diastolic dysfunction in patients with diastolic heart failure [21, 22]. The reasons that contributed for these contrasting results are not clear, but could be due to differences in inclusion criteria, intervention protocols, and duration of exercise training. For example, Smart *el al.* did not include a control group [21]. Thus, the evolution of cardiac function in exercising patients could not be compared with that in patients that are not submitted to exercise training. Furthermore, patients included in the study by Kitzman *et al* [22] were older (70 ± 6 yrs) and exercised for a shorter period of time (16 weeks) compared with our patients. Thus, longer exercise training programmes could be necessary to improve cardiac function in older individuals. On the other hand, in the same study [22] both exercise and usual care groups changed diastolic function and decreased ejection fraction over time. Thus, experimental procedures or pharmacological intervention could account for these observations.

The present data also indicate that endurance training improves exercise tolerance in patients with HFPEF. These observations are line with those documented in previous studies [22, 25, 26]. This is an important finding, because these patients are usually advanced in age and because exercise capacity is a strong determinant of health-related quality of life [11]. The mechanisms of exercise intolerance remain unclear in HFPEF. It has been suggested that abnormal increases in left ventricular end-diastolic pressures during exercise and reduced preload reserve are important contributors to exercise intolerance in HFPEF [16, 27]. Moreover, chronotropic incompetence is present in many patients with HFPEF [18]. In the present study, exercise training

improved diastolic function, decreased resting heart rate and increased heart rate reserve, all of which may have contributed to enhance diastolic filling time, diastolic reserve and exercise tolerance [18, 28]. Minor improvements in resting left ventricular systolic function were also noticed following exercise training, but it is not possible to determine its contribution to exercise tolerance. Previous studies have demonstrated that blunted increases in cardiac output, mitral annular systolic velocity, and cardiac contractility during exercise are associated with exercise intolerance in patients with HFPEF [12, 29, 30]. However, these studies have measured contractile function during exercise or used more subtle measures of contractile function. Thus, the present data cannot determine the contribution of exercise-induced improvements in left ventricular systolic function to exercise tolerance in patients with HFPEF. Other mechanisms for improved exercise tolerance could include changes in endothelial function, peripheral vascular resistance, cardiac metabolism and skeletal muscle functional, histological and metabolic characteristics [31-33], as recent evidence indicates that all these elements may be abnormal in patients with HFPEF [17, 34].

The present study has a number of limitations. Although one could advocate that the sample size seems relatively small, we remind that we have estimated it based on studies that tested the effects of exercise training in patients with similar characteristics as ours [22, 25]. We calculated the outcome based on a difference that was above the clinical threshold of exercise improvement (10%), and we have achieved a similar total sample size as that of reference studies [22, 25]. In addition, it is not possible to provide data concerning the reproducibility of left ventricular function and dimensions. Nonetheless, since all echocardiographic evaluations were performed by the same physician, and given his blindness to the treatment assignment, we are confident that evaluation bias has not influenced the outcomes of this investigation. We used exercise grade and speed on a treadmill test as an indicator of improved functional capacity. It has been shown that a learning effect can occur with exercise duration and the subjects did not perform a familiarization exercise test to overcome this issue. On the other hand, only one assessor supervised all exercise tests and he was unaware of the treatment assignment, avoiding assessment biases or inter-observer variability. In addition, the slight decrement

observed in the control group indicates that the differences between groups reflect true changes in exercise tolerance and not simply a learning effect.

In summary, the present data indicates that exercise training may improve left ventricular diastolic dysfunction, exercise hemodynamics and exercise tolerance in patients with HFPEF. These changes induced by exercise training may serve to enhance left ventricular diastolic filling time, diastolic reserve and exercise tolerance in patients with this condition. These results suggest that exercise training should be considered as an effective non-pharmacological treatment to improve symptomatic status of heart failure patients.

Acknowledgements

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Study II

Exercise training improves diastolic function in heart failure patients

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Abstract

Purpose: To analyze the effects of exercise training on exercise tolerance, left ventricular systolic function and structure in heart failure patients with preserved, mild and moderate to severe reduction of left ventricular ejection fraction (LVEF).

Methods: Ninety-eight patients with moderate to severe (n= 34), mild (n= 33) and preserved (n= 31) LVEF were randomly assigned to exercise training plus usual care (n=65) or usual care alone (n=33) in a randomization ratio of 2:1. Left ventricular function, left ventricular dimensions, and exercise tolerance were assessed before and after each intervention.

Results: Exercise tolerance and LVEF increased with exercise training in all patient groups, while they remained unchanged after usual care alone. Exercise training increased the mean ratio of early to late mitral inflow velocities (E/A ratio) and decreased deceleration time of early filling (DT) in patients with mild and preserved LVEF. In patients with moderate to severe systolic dysfunction and advanced diastolic dysfunction (DT<160 ms), exercise training decreased E/A ratio and increased DT, both of which were unchanged after usual care alone. In the remaining patients (DT>160 ms), exercise training also improved mitral inflow patterns. Exercise training decreased left ventricular dimensions in patients with mild and moderate to severe reduction of LVEF, but not in patients with preserved LVEF.

Conclusions: These results indicate that exercise training can improve the course of heart failure independent of the degree of baseline left ventricular dysfunction.

Keywords: cardiac failure, ejection fraction, chronic exercise, usual care, echocardiography

Introduction

It is well established that clinical presentations are similar in heart failure patients with reduced and preserved ejection fraction. A number of studies have shown that exercise tolerance is reduced to the same extent in heart failure with reduced ejection fraction (HFREF) as it is in heart failure with preserved ejection fraction (HFPEF) (20, 24). Moreover, mounting evidence indicates that left ventricular diastolic function is impaired in patients with heart failure, independent of ejection fraction (39, 40), and that contractile abnormalities can be present in patients with HFPEF despite preserved left ventricular systolic function (11, 37). On the other hand, several differences are evident in left ventricular functional and structural characteristics in patients with HFREF and HFPEF. Increased left ventricular stiffness in tandem with concentric remodeling and increased left ventricular mass are common features in HFPEF. This contrasts with left ventricular dilation and resting contractile dysfunction, which are more common in HFREF (11). The unequal remodeling characteristics suggest that outcomes can be distinct despite similar treatment, as the positive outcomes in clinical trials with HFREF have contrasted with the neutral results recently reported in HFPEF (28).

It is well known that exercise training improves exercise capacity and quality of life in patients with heart failure (26). Ample evidence supported in recent meta-analyses indicates that exercise training can also improve left ventricular function, decrease dilatation and reduce hospital admissions in patients with HFREF (9, 15), but little is known in patients with HFPEF (18). Indeed, it has never been shown whether exercise training can improve diastolic dysfunction in patients with HFPEF. Given the association of mitral filling patterns with functional class and prognosis in patients with heart failure (12, 29), most studies hitherto have used mitral inflow velocities and time intervals to measure the impact of exercise training on diastolic dysfunction (4, 18). However, it is unclear whether mitral inflow velocities have a uniform response to exercise training, because patients with different levels of dysfunction show distinct filling patterns (22). For example, early (E) mitral flow has a prolonged deceleration time and its contribution relative to atrial (A) mitral flow to ventricular filling is reduced in patients with mild diastolic dysfunction, whereas E mitral flow becomes predominant with rapid deceleration in patients with more severe diastolic

dysfunction (22). These unique filling patterns suggest that exercise training may require unique responses to improve diastolic dysfunction in patients with different levels of diastolic dysfunction. Given the hypothesis that left ventricular functional and structural differences can lead to different responses to treatment and exercise in patients with reduced and preserved ejection fraction, this study aimed to investigate the effects of exercise training on exercise tolerance, left ventricular function and structure in heart failure patients with preserved, mild and moderate to severe reduction of ejection fraction.

Methods

Patient recruitment

Patients admitted to Bnai Zion Medical Center, Haifa, Israel, were considered eligible to participate in the study if they presented signs or symptoms of heart failure. Exclusion criteria included uncontrolled hypertension, unstable angina pectoris, abnormal hemodynamic response, uncontrolled cardiac arrhythmias, ischemic electrocardiogram changes during stage one of exercise tolerance test (Modified Bruce protocol), uncontrolled metabolic disease (e.g., uncontrolled diabetes and thyroid disease) and medical conditions that limit participation in exercise (e.g., peripheral arterial occlusive disease and musculoskeletal disorder). The hospital ethics committee approved the study protocol and written informed consent was obtained from every patient.

Study design

Subjects were randomly assigned to exercise training plus usual care or usual care alone (control group) in a randomization ratio of 2:1. Twice as many patients were assigned to the exercise-training group to compensate for the attrition and lower adherence in patients with heart failure who participate in cardiac rehabilitation programs (3). Patients who completed less than 80% of exercise sessions were excluded from the analysis. Usual care consisted of regular appointments with a cardiologist and optimized medication. Patients in control group did not receive instructions or any form of exercise training. Patients in both groups were assessed at baseline and 6 months after randomization (follow-up).

Subjects were classified into three patient groups based on baseline left ventricular ejection fraction (LVEF), according to the recommendations of the American Society of Echocardiography (21): preserved (>55%), mild (from 45% to 54%) and moderate to severe (<45%) reduction of LVEF.

Exercise Testing

Each subject performed a symptom limited, graded exercise treadmill test according to a standard Modified Bruce protocol. To examine exercise tolerance, we recorded treadmill exercise time and estimated metabolic equivalents (METs) according to the guidelines provided by the American College of Sports Medicine (1):

MET = [(speed x 0.1) + (grade x speed x 1.8) + 3.5] / 3.5, where fractional grade is expressed in decimal form and speed in meters per minute (1 mile per hour is equal to 26.8 meters per minute).

The percentage of change in exercise tolerance (in METs) was calculated as follows: (Follow-up METs – Baseline METs) / Baseline METs x 100. A clinically meaningful improvement in exercise tolerance was judged to a 10% increase from baseline to follow-up METs, as previously described (18).

Blood pressure (by auscultation) and a 12-lead electrocardiogram were recorded at rest, throughout exercise, and at regular intervals during recovery until heart rate and electrocardiogram had returned to baseline (recovery data not used for this study). Maximal heart rate was determined as the highest heart rate achieved during the last 30 seconds of exercise testing. Treadmill tests were terminated according to the guidelines recommended by the American College of Sports Medicine (1). The same cardiologist, who was blinded to the treatment group, supervised all treadmill tests.

Echocardiographic evaluation

All subjects underwent a complete resting echocardiography examination using a Siemens-Acuson Sequoia machine (USA). Three consecutive cardiac cycles were analyzed and averaged for each patient. LVEF was measured using the modified biplane Simpson's method from the apical 4- and 2-chamber views, whereas left ventricular end-diastolic diameter (EDD) and left ventricular end-systolic diameter (ESD) were measured at M-mode in the parasternal long-axis view. Transmitral inflow velocities were assessed by pulsed-wave Doppler, with the sample volume placed

between the mitral leaflet tips in the apical 4-chamber view. The peak early (E) and late (A) transmitral velocities and the E-wave deceleration time (DT) of early filling velocity were assessed and the E/A ratio was calculated for the evaluation of diastolic function. One cardiologist performed all echocardiographic evaluations and was blinded to the treatment group.

Exercise Training

Patients trained three times a week for 6 months. In the first month, each exercise session consisted of 10 min warm-up exercises, 15 min of aerobic exercise and 10 min of cool-down with stretching exercises. The aerobic exercise consisted of interval training performed on a treadmill or bicycle ergometer - 5 sets of 3 min exercise at 70-75% of maximal heart rate interspersed with 1 min active recovery at 45-55% of maximal heart rate. In the following five months, aerobic exercise duration was progressively increased up to 35 minutes by increasing the number and duration of exercise sets (7 x 5 min), while maintaining the duration of active recovery. Heart rate was continuously monitored by ECG with each minute rhythm strip hard copy recorded. Exercise intensity was adjusted progressively throughout the study to ensure that all exercise-training sessions were performed within the established heart rate.

Statistical Analysis

Normal data distribution was confirmed by the D'Agostino & Pearson omnibus test and Shapiro-Wilk normality test. Data are presented as mean ± SD unless otherwise stated. Continuous variables with non-normal distribution were log-transformed before statistical testing and means were transformed back for presentation. To examine the effect of treatments on exercise tolerance, cardiac function and left ventricular dimensions, a two-way mixed-model ANOVA was used to compare results between treatment groups over time (treatment x time). When significant interactions were observed, *t*-tests were applied to determine the location of differences within each treatment relative to baseline as well as between treatments at baseline and follow-up. The association between treatments and the proportion of patients who improved exercise tolerance above the clinical threshold (10%) was examined through contingency tables with chi-square statistic. To account for the inflation of type I error that may occur with multiple comparisons, statistical significance was corrected with

Holm-Bonferroni method where appropriate. Mean differences were adjusted for potential confounders where appropriate. $P < 0.05$ was considered indicative of statistical significance.

The estimation of sample size was based on previous research (15), which has demonstrated that exercise training induces a moderate effect on left ventricular function, while the control group is expected to have no change. This analysis revealed that based on the randomization ratio of 2:1, 19 patients in the exercise training and 9 in the control group, in each patient group would be required to detect, a time x treatment interaction with a moderate effect size (f = 0.25) in left ventricular function with 80% of probability, at a α-level of 0.05. The total number of patients was inflated to account for patients who would not be able to complete the programme or would be unable to perform the follow-up evaluations (drop-outs) (3).

Results

One hundred three patients were eligible and agreed to participate in this study. Among patients from the exercise-training group (n=67), 1 was unable to attend the exercise programme and 1 did not perform the follow-up echocardiographic evaluation. Of the patients who received usual medical care and constituted the control group (n=36), 1 was unable to attend both echocardiographic evaluations while 2 patients were unable to attend the echocardiography follow-up evaluation. Thus, a total of 98 patients completed the study and were included in the analysis. The ratio of patients assigned to exercise training and usual care alone was similar in all patient groups, that is 22 versus 12 patients in the moderate to severe group, 23 versus 10 patients in the mild group, and 20 versus 11 patients in the preserved ejection fraction group. No adverse events were registered during exercise testing or during aerobic interval training. Our patients tolerated the exercise training protocol and did not have any problems in maintaining their target heart rate range throughout the exercise sessions.

Baseline Characteristics

Baseline characteristics are shown in Table 1. There was a greater proportion of women and a lower prevalence of prior myocardial infarction in patients with

preserved LVEF. On the other hand, there were more patients treated with angiotensin-converting enzyme (ACE) inhibitors and spironolactone in the moderate to severe group compared with the other patient groups. No significant differences were found between exercise training and usual care alone in any patient group with respect to demographical, cardiovascular risk factor profile and medication regime characteristics.

Table 1. Demographic and clinical characteristics of patients at baseline

	Moderate to Severe (N=34)	Mild (N=33)	Preserved (N=31)
Age (yrs)	62.0 ± 9.9	63.6 ± 10.9	62.9 ± 10.2
Sex (M/F)	27/7	24/9	22/9
BMI (kg/m2)	28.5 ± 4.4	29.5 ± 4.8	28.4 ± 4.5
NYHA			
I	1 (2.9%)	3 (9%)	2 (6.5%)
II	11 (32.4%)	15 (45.5%)	12 (38.7%)
III	21 (61.8%)	15 (45.5%)	17 (54.8%)
IV	1 (2.9%)	0 (0%)	0 (0%)
STEMI	12 (35%) *	8 (24%)	6 (19%)
NSTEMI	15 (44%) *	12 (36%)	4 (13%)
Dilated cardiomyopathy	4 (12%)	0 (0%)	0 (0%)
Atrial Fibrillation	0 (0%)	1 (3%)	1 (3%)
Hypertension	22 (65%)	25 (76%)	21 (68%)
Diabetes Mellitus	10 (29%)	12 (36%)	11 (35%)
Beta-blockers	24 (70%)	28 (85%)	20 (74%)
ACE inhibitors	17 (50%) *	6 (19%)	10 (32%)
AT1 antagonists	7 (20%)	8 (24%)	6 (19%)
Calcium channel blockers	3 (8%)	8 (24%)	7 (29%)
Diuretics	9 (26%)	5 (15%)	5 (16%)
Statins	26 (76%)	28 (85%)	23 (74%)
Spironolactone	5 (15%) †	0 (0%)	2 (6%)
LVEF (%)	37.3 ± 7.9 ‡	49.3 ± 1.9	56.3 ± 2.5
METs	3.6 ± 1.1	4.3 ± 1.4	3.9 ± 1.4

BMI, Body mass index; NYHA, New York Heart Association; STEMI, ST elevation myocardial infarction; NSTEMI, Non-ST elevation myocardial infarction; ACE, Angiotensin converting

enzyme; AT1, Angiotensin-II type 1 receptors; LVEF, Left ventricular ejection fraction; METs, Metabolic equivalents. Data are presented as mean ± SD and absolute frequency (percentage). ∗ Significantly different than mildly reduced and preserved LVEF groups. P<0.01; † Significantly different than mildly reduced and preserved LVEF groups. P<0.05; ‡ Significantly different than mildly reduced and preserved LVEF groups. P<0.001

Left ventricle function

LVEF increased after exercise training in all patient groups (Figure 1), while it remained unchanged with usual care alone (treatment x time interaction in preserved: $F = 6.33$, $P = 0.02$; mild: $F = 15.53$, $P < 0.01$; moderate to severe: $F = 28.78$, $P < 0.001$).

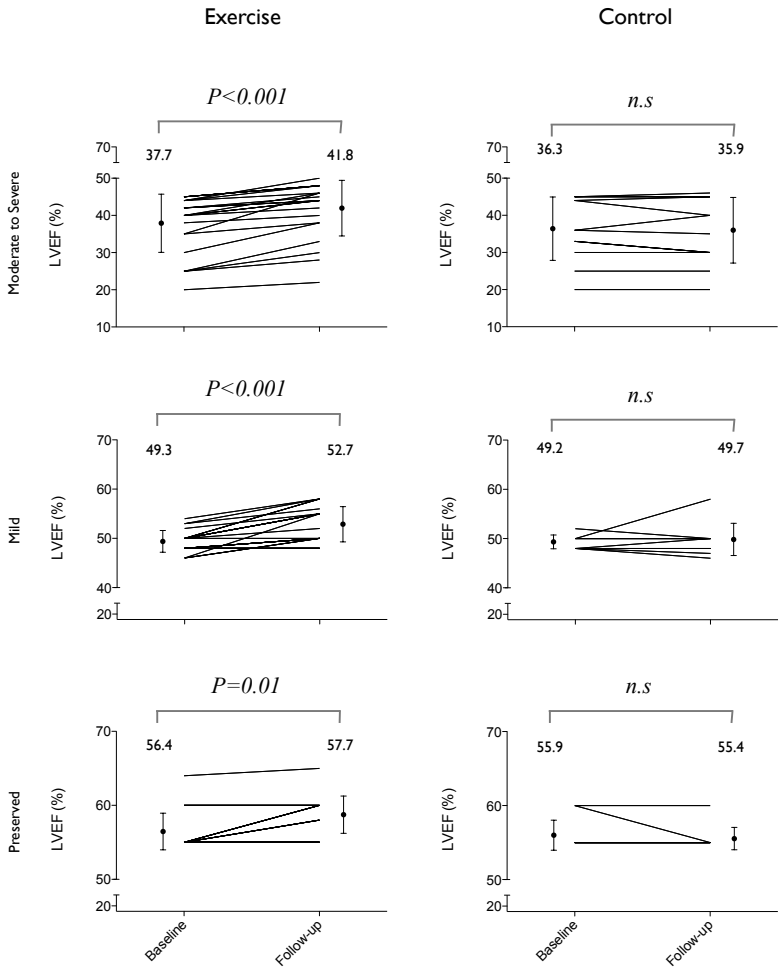

Figure 1. Baseline and follow-up values of left ventricular ejection fraction (LVEF) in exercise training and control groups.

Diastolic function improved after exercise training but not after usual care alone in patients with preserved (treatment x time interaction: $F = 4.73$, $P = 0.02$) and mild systolic dysfunction (treatment x time interaction: $F = 3.97$, $P = 0.03$). The E/A ratio increased after exercise training in both patient groups, while it remained unchanged with usual care alone (Figure 2a). Furthermore, the E-wave deceleration time (DT) decreased after exercise training in both patient groups, while it remained unaltered following usual care alone (Figure 2b).

When the data was pooled, diastolic function was similar between exercise training and usual care alone groups in patients with moderate to severe systolic dysfunction (Figure 2). However, among these patients there were different degrees of diastolic dysfunction, which led us to conduct separate analysis in patients that presented a short deceleration time (DT<160 ms) (25), indicating severe diastolic dysfunction (restrictive filling), and patients that presented prolonged deceleration time (DT>160 ms), indicating mild diastolic dysfunction (impaired relaxation). In patients with severe diastolic dysfunction, exercise training increased the short deceleration time towards normal (from 129.8 ± 6.1 to 166.6 ± 11.8 ms, P <0.001) and promoted a significant decrease in the E/A ratio (from 1.58 ± 0.11 to 1.24 ± 0.22, P =0.02), while in patients with mild diastolic dysfunction, exercise training decreased the prolonged deceleration time (from 243.5 ± 37.0 to 221.5 ± 19.0 ms, $P < 0.001$) and enhanced the E/A ratio towards normal (from 0.79 ± 0.11 to 0.90 ± 0.08, $P < 0.001$). No such changes were observed in the usual care alone group.

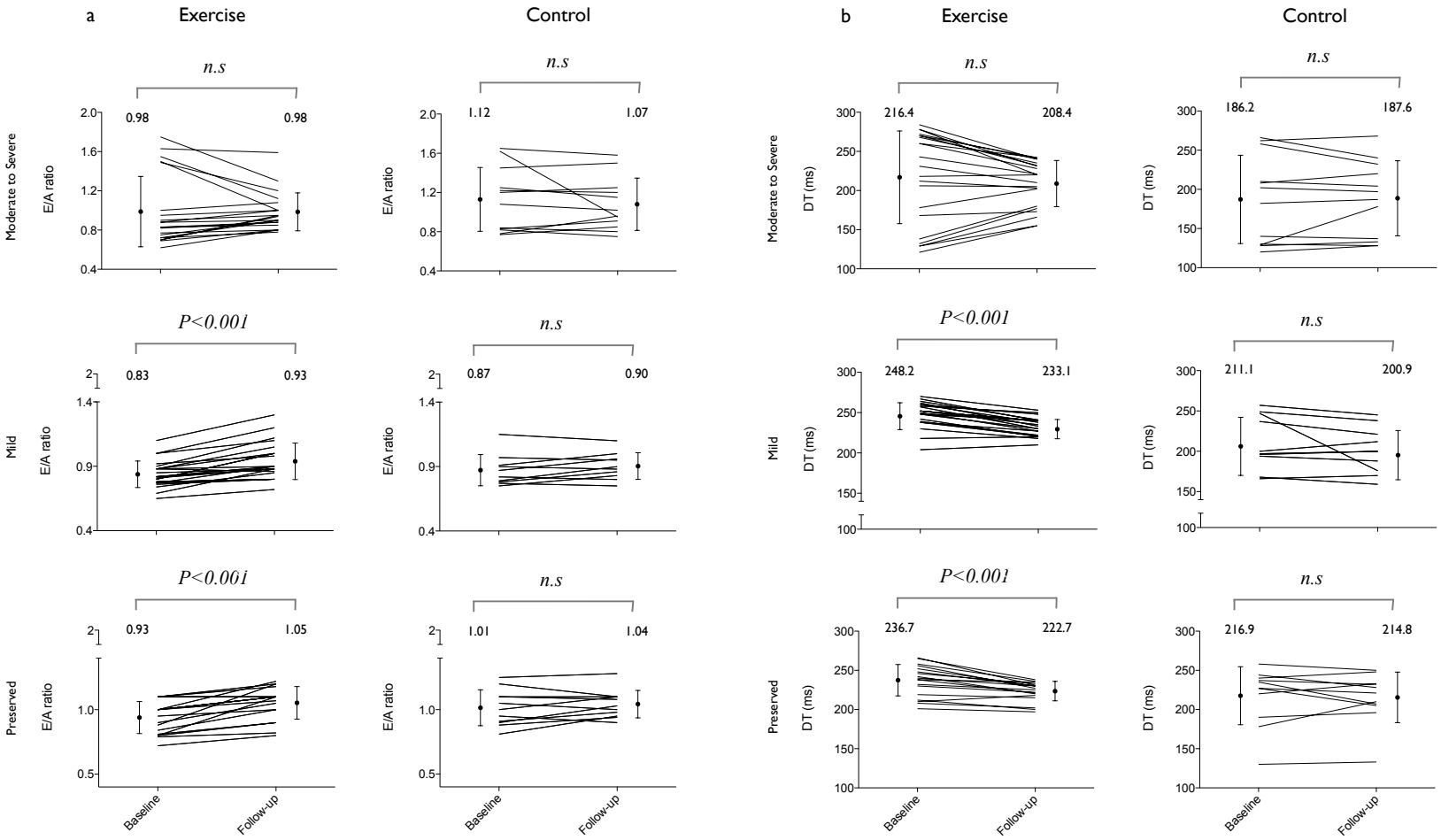

Figure 2. Baseline and follow-up values of E/A ratio (a) and DT (b) in exercise training and control groups.

Left ventricle dimensions

Left ventricular dimensions were altered by exercise training in the mild and moderate to severe patient groups ($F = 3.75$, $P = 0.035$ and $F = 9.39$, $P < 0.01$, Figure 3) but not in patients with preserved systolic function. In patients with moderate to severe systolic dysfunction, left ventricular dimensions decreased following exercise training, while this was not observed in the control group. In contrast, in patients with mild systolic dysfunction, exercise training promoted a decrease in ESD ($p<0.001$), but not in EDD, while usual care alone had no impact on left ventricular diameters.

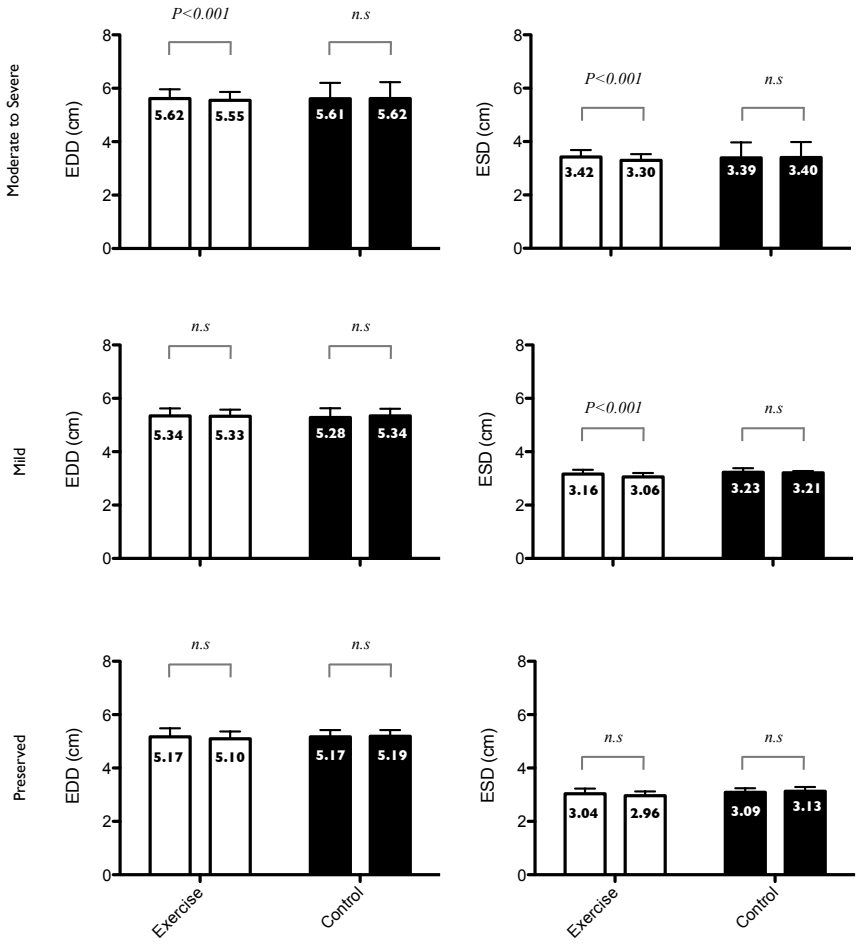

Figure 3. Baseline and follow-up left ventricular dimensions in exercise training and control groups. EDD, end-diastolic diameter; ESD, end-systolic diameter.

Exercise tolerance

Baseline and follow-up exercise tolerance are shown in Figure 4. Exercise tolerance increased in all exercise groups, whereas it remained unchanged in all usual care alone groups (treatment x time interaction: preserved: $F = 4.81$, $P = 0.04$; mild: $F = 6.17$, $P = 0.02$; moderate to severe: $F = 4.07$, $P = 0.05$). Moreover, the number of patients with a 10% improvement in exercise tolerance, conventionally used as clinically relevant, was higher in all exercise patient groups when compared with the usual care alone groups (moderate to severe: 62% vs. 22%, $\chi^2 = 3.96$, $P = 0.04$; mild: 48% vs. 11%, $\chi^2 = 3.61$, $P = 0.05$; preserved: 45% vs. 0%, $\chi^2 = 7.51$, $P < 0.01$).

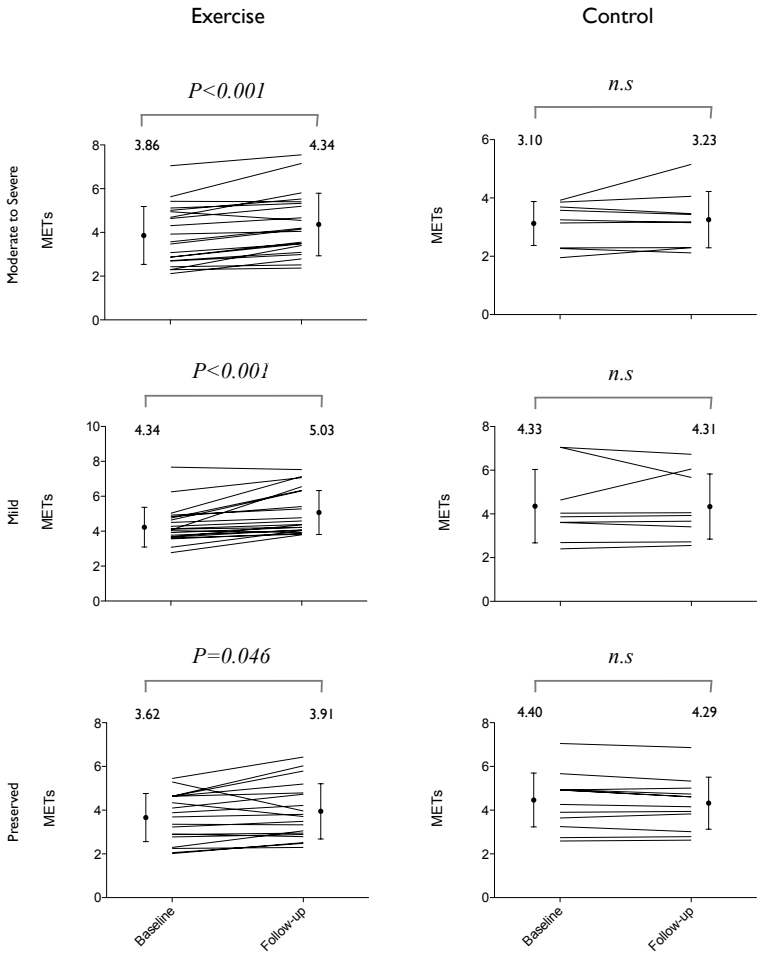

Figure 4. Baseline and follow-up values of exercise tolerance in exercise training and control groups.

Discussion

The main findings of the present study were that exercise training improved exercise tolerance and cardiac function in patients with moderate to severe, mild and preserved left ventricular ejection fraction (LVEF), with all those receiving usual medical care alone remaining unaltered.

It is well known that exercise training can improve systolic function in patients with heart failure and reduced ejection fraction (HFREF) (15). In this study, we showed that left ventricular performance could also improve in heart failure patients with near normal and normal LVEF (HFPEF). These results are intriguing, because left ventricular function and its driving mechanisms differ in HFREF and HFPEF. The preserved LVEF indicates that left ventricular performance is well maintained, but recent studies have shown that myocardial contractile dysfunction can be present in patients with HFPEF (11). For example, recent evidence demonstrated that systolic mitral annular velocity and left ventricular longitudinal shortening are reduced in patients with HFPEF compared to controls (7, 34). It is speculated that the reasons for the depressed contractile performance in these patients are concentric remodeling and ventricular stiffening, resulting in reduced myocardial contractility and systolic reserve (6, 39). Indeed, although systolic function is arguably not as impaired in HFPEF as in HFREF, recent studies have shown that mild limitations in resting contractile function can become quite limitative during physical exertion (5, 24). Exercise training did not change concentric remodeling in our patients with HFPEF, suggesting that it might improve contractile function by reducing ventricular stiffening. Numerous factors determine myocardial stiffness, including the expression of different isoforms and phosphorylation status of cytoskeletal protein titin, phosphorylation of sarcomeric proteins and the amount, distribution and architecture of fibrillar collagen in the extracellular matrix (6, 22). Evidence from experimental studies in animals indicates that exercise training can reduce collagen volume fraction after myocardial infarction (36), but the consequences of exercise training to the expression and phosphorylation of myofilament and cytoskeletal proteins remain unclear. An alternative candidate mechanism is the restoration of β-adrenergic intracellular signaling transduction following exercise training (10). This could improve contractile dysfunction related to receptor downregulation and abnormal

calcium metabolism on one hand (32), and reduce cardiomyocyte stiffness through the phosphorylation of cytoskeletal (titin) or sarcomeric proteins on the other (33). It should be noted that changes in left ventricular loading conditions and chamber geometry have a significant influence on LVEF. However, left ventricular end-diastolic dimensions did not change with exercise training in our patients with mild systolic dysfunction and HFPEF, supporting the notion that left ventricular contractile function may have improved in these patients.

Another main finding of this investigation was that mitral inflow velocities and filling patterns improved in all exercise training groups, remaining unchanged after usual care alone. This is an important finding because mitral inflow velocities and filling patterns are associated with functional class and prognosis in heart failure patients (12). In contrast to normal patients, the E-wave velocity during left ventricular relaxation is reduced in association with a prolonged deceleration time in patients with mild systolic dysfunction (impaired relaxation) (22). When diastolic pressures increase such that atrial contraction cannot increase left ventricular filling, the E-wave becomes predominant again in tandem with a rapid deceleration time, indicating severe diastolic dysfunction (22). Severe diastolic dysfunction is associated with poor prognosis, especially if the restrictive filling pattern persists after treatment (29). In this study, exercise training improved diastolic dysfunction in patients with impaired relaxation as well as advanced diastolic dysfunction in a number of patients with heart failure and moderate to severe systolic dysfunction. The E-wave velocity contribution to left ventricular filling increased after exercise training in patients with impaired relaxation, whereas it decreased towards normal in patients with restrictive filling pattern. Moreover, deceleration time returned to values towards normal in both groups following exercise training, independent of its initial value. These results are consistent with a number of previous studies in HFREF (4, 35), but are in contrast with those reported in two recent studies (18, 31), in which diastolic function did not improve with exercise training in patients with near normal and normal LVEF. This discrepancy is likely to be due to differences in sample size, inclusion criteria, definition of normal ejection fraction and exercise training protocol. Another important element to retain from these data is that exercise training seems to have an extensive mechanistic action, as the causes of diastolic dysfunction may not be the

same in patients with HFREF and HFPEF. It has been proposed that reduced elastic recoil due to impaired contractile function and left ventricular dilatation decrease intraventricular pressure gradient in patients with HFREF (11). Our data indicate that both elements improve in these patients with exercise training. In contrast, the end-diastolic pressure-volume relation shows an inappropriate upward and leftward shift in patients with HFPEF, indicating increased filling pressures associated with left ventricular stiffness (2). Our results indicate that end-diastolic dimensions remain unaltered with exercise training in patients with HFPEF, leaving unclear whether it has any impact on left ventricular passive stiffness. On the other hand, left ventricular relaxation is impaired in both subsets of heart failure (38), indicating abnormalities in calcium handling or phosphorylation of sarcomeric proteins (22). There is indeed evidence from experiments in animals that exercise training normalizes calcium handling in tandem with the expression of SERCA2a and phosphorylation status of phospholamban (17), and that it restores the phosphorylation of sarcomeric proteins, in particular Myosin Light Chain 2 (10). It is important to take into consideration that mitral flow parameters are affected by left ventricular loading conditions. Numerous factors can mediate alterations in loading conditions induced by exercise training. An obvious and intuitive suggestion might be neurohormonal activation, as it is elevated in all patients with heart failure with increased sympathetic activation and circulating catecholamines (20). Indeed, exercise training improves baroreflex sensitivity and decreases the activation of sympathetic nervous system in patients with HFREF (30). This decreases afterload and reduces peripheral vascular resistance, all of which help to improving cardiac function and decrease remodeling.

Exercise intolerance, manifested by premature fatigue and dyspnea during physical exertion, is the prime manifestation of heart failure. It is associated with reduced quality of life and increased mortality (16). It is well known that exercise training improves exercise tolerance in patients with moderate to severe systolic dysfunction (26). In the present study, most patients in all exercise-training groups improved exercise tolerance above the limit that is considered clinically relevant, which was not observed with usual care alone. However, our data extended the previous observations (18, 26) by showing that exercise tolerance improved more pronouncedly in patients with moderate to severe systolic dysfunction compared with the other groups. These

observations suggest that exercise training should be considered as a fundamental non-pharmacological treatment in heart failure, eventually with greater benefits in patients with worse prognosis. Nevertheless, this requires further investigation.

Despite these findings, the literature thus far cannot elucidate which are the mechanisms by which exercise training improves exercise tolerance in different subsets of heart failure. It is complex to discern the importance of cardiac function to exercise tolerance in different subsets of heart failure, because left ventricular function and structure differ in patients with reduced and preserved ejection fraction. Nonetheless, left ventricular function improved in all exercise-training groups, in parallel with exercise tolerance, and remained unchanged with usual care alone, suggesting that exercise training improves exercise tolerance in concert with cardiac function. Numerous studies have postulated that exercise tolerance is weakly associated with left ventricular function (19), but a limitation of most exercise studies, as well as ours, is that mechanistic measurements have been made only at rest. Our data indicate that exercise training improves left ventricular filling dynamics in all subsets of heart failure. It is proposed that increased left ventricular pressures associated with a small preload reserve lead to exercise intolerance, resulting from failure to increase stroke volume and increased pulmonary venous pressure (23, 27). By improving left ventricular filling dynamics, exercise training could decrease pulmonary vascular resistance and increase stroke volume during exercise. This could in turn attenuate the appearance of dyspnea and improve exercise tolerance.

Besides these mechanisms, studies in patients with dilated and ischemic cardiomyopathies reported that exercise training improves endothelial dysfunction and decreases vascular resistance (13, 14), resulting in improved ventricular-vascular responses during exercise and in improved exercise capacity (13, 14). Although the exercise mechanistic role remains to be established, mounting evidence indicates that patients with normal or near normal systolic dysfunction also have abnormal vasomotor and ventricular-vascular coupling responses during exercise (5), and could benefit from exercise training. Moreover, the importance of functional, metabolic and histological changes in skeletal muscle to improving exercise tolerance in patients with HFREF has been widely recognized (8). However, this remains to be elucidated in patients with mild systolic dysfunction and preserved ejection fraction.

Limitations

A limitation of this study is that we have used exercise grade and speed on a treadmill test as the indicator of improved functional capacity, instead of a direct measure of exercise capacity such as oxygen consumption. A few studies have shown that a learning effect may occur with exercise duration and, indeed, our subjects did not perform a familiarization exercise test in order to overcome this issue. However, the designated assessor was blinded to the treatment groups, and some of the control groups showed slight declines in exercise tolerance, indicating that the differences between groups reflect true changes in exercise capacity and not a learning effect.

It can be argued that sample size is small in all groups, in particular those pertaining to controls. However, estimates from previous literature indicate that our sample sizes are large enough to detect differences in exercise tolerance and left ventricular function induced by exercise training and control groups, and avoid type I errors with 80% certainty.

Patient management, exercise prescription and implications for future research

Exercise intolerance is the cardinal manifestation of heart failure, and it is common to all heart failure patients. The present findings showed that exercise training improves exercise tolerance and left ventricular function in heart failure patients independent of their baseline condition. Thus, it is recommended that exercise training be included in the treatment of all heart failure patients. The question that remains open is which mechanisms are used by exercise training to improve left ventricular function and exercise tolerance in patients with HFREF and HFPEF. Indeed, the effects of exercise training on the cellular and molecular mechanisms of left ventricular stiffness and active relaxation and their association with systolic and diastolic reserve represent promising avenues of research in the future. Future studies should also investigate whether exercise training improves endothelial dysfunction, ventricular-vascular coupling responses to exercise and skeletal muscle characteristics in patients with HFPEF.

Our results also indicate that moderate intensity interval training offers a sufficiently strong stimulus to improve exercise tolerance and cardiac function. High intensity exercise training has been shown to have a superior cardiovascular effect in patients with HFREF (35). However, it remains to be determined if high intensity exercise training interferes with the safety and compliance of heart failure patients compared to moderate exercise training programs.

Summary

The present study showed that exercise training could improve exercise tolerance and left ventricular function in heart failure patients, independent of their baseline systolic dysfunction. These results are relevant because they indicate that exercise training can improve the course of heart failure independent of the degree of baseline left ventricular dysfunction.

Acknowledgements

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Study III

GNAS A-1121G variant is associated with improved diastolic dysfunction in response to exercise training in heart failure patients.

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Abstract

Background: β1-adrenergic receptors (*ADRB1*) and Gαs proteins (*GNAS*) play important roles in the regulation of cardiac function. **Aims:** The present study sought to investigate whether *ADRB1* Arg389Gly (rs1801253), *GNAS* -1211 G/A (rs6123837) and *GNAS* 2291 C/T (rs6026584) variants are associated with left ventricular function and exercise tolerance in heart failure patients. **Methods:** Sixty-one heart failure patients completed a 6-month exercise-training programme. Left ventricular ejection fraction (LVEF), mitral inflow velocities (deceleration time and E/A ratio) and exercise tolerance (METs) were assessed at baseline and following exercise training. **Results:** There were no associations between the studied variants and LVEF or E/A ratio measured at baseline and after exercise training. Deceleration time of early mitral flow was higher at baseline in *GNAS* -1211G allele carriers compared with -1211A allele homozygotes ($P<0.05$). Exercise training attenuated deceleration time in -1211G allele carriers ($P<0.05$) but not in -1211A allele homozygotes. Moreover, ADRB1 389Gly homozygotes had a greater training-induced increase in exercise tolerance than 389Arg homozygotes ($P=0.04$). **Conclusions:** This study shows that the functional *GNAS* -1121 G/A polymorphism is associated with improved diastolic function following exercise training in heart failure patients. Furthermore, our data suggest that *ADRB1* Arg389Gly polymorphism influences exercise tolerance.

Keywords: exercise, polymorphism, heart failure

Introduction

β1-adrenergic receptors, encoded by *ADRB1* gene play an important role in the regulation of cardiac function [36]. When stimulated with catecholamines, these receptors couple with stimulatory Gα proteins (encoded by *GNAS* gene) and activate adenylate cyclase. This results in the phosphorylation of calcium regulatory proteins, promoting an increase in cardiac contractility and acceleration of cardiac relaxation [42].

Heart failure patients have increased sympathetic activity and elevated circulating catecholamine levels [27, 29]. In addition, maximal adenylate cyclase activity and muscle contraction are reduced in failing human hearts compared with normal hearts [6, 7]. Failing human hearts have also shown reduced contraction and relaxation rates in tandem with abnormalities in calcium regulatory proteins [13, 34]. These functional changes are associated, at least in part, with abnormalities in β1 - adrenergic receptor intracellular signalling [6, 17].

Numerous gene variants have been associated with clinical outcomes in heart failure patients [1]. In the *ADBR1* gene, a substitution of an arginine to a glycine at codon 389 (Arg389Gly) in the receptor cytoplasmic tail has been shown to alter the receptor coupling [32]. Both *in vitro* and *in vivo* studies have demonstrated that the Arg389 variant is associated with an increased agonist-stimulated adenylate cyclase activity and increased cardiac contractility compared with the Gly389 variant [32, 33, 37]. On the other hand, the Arg389 variant is more prone to receptor desensitization when exposed to increased concentrations of catecholamines [33, 35]. Variants located in the promoter region and in intron 1 of the *GNAS* gene have also been associated with increased basal and agonist-stimulated adenylate cyclase activities, as well as with increased cardiac contractility in coronary artery disease patients [18]. However, to our knowledge, the association between *GNAS* variants and left ventricular function is yet to be determined in heart failure patients.

Exercise training has been shown to improve exercise tolerance and left ventricular function in heart failure patients [22, 41]. Improvements in sympathetic tone and adrenergic responsiveness were also observed following endurance training [12, 38]. Previous studies have shown that the Arg389Gly polymorphism is associated with

exercise tolerance during exercise testing in both heart failure [40], and coronary artery disease patients [15]. However, the relationship between *ADRB1* and *GNAS* genes and exercise tolerance in response to aerobic exercise training is unclear in heart failure patients. Thus, the aim of the present study was to test the association between *ADRB1* Arg389Gly (rs1801253), *GNAS* -1211 G/A (rs6123837) and *GNAS* 2291 C/T (rs6026584) variants and left ventricular function and exercise tolerance in heart failure patients that underwent an interval exercise training program.

Methods

Subjects

Sixty-seven heart failure patients in NYHA (New York Heart Association) class I-III with either preserved or reduced left ventricular ejection fraction (HFPEF & HFREF) were included in the study as previously described [2]. Exclusion criteria included uncontrolled hypertension, unstable angina pectoris, uncontrolled cardiac arrhythmias, significant ischemic electrocardiogram ST-T changes during the initial stages of the exercise tolerance test (Modified Bruce protocol), uncontrolled metabolic disease (e.g., uncontrolled diabetes and thyroid disease) and medical conditions that limit exercise participation (e.g. peripheral arterial occlusive disease and musculoskeletal disorder). The ethics committee of the Bnai-Zion Haifa Medical Center, Israel, approved the study protocol and written informed consent was obtained from every patient. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki. The study has moreover been conducted in accordance with the ethical standards of the IJSM [23].

Exercise Testing

Each subject performed a symptom limited, graded exercise treadmill test according to a standard Modified Bruce protocol. To examine exercise tolerance, treadmill exercise time was recorded and metabolic equivalents (METs) estimated according to the guidelines provided by the American College of Sports Medicine:

MET = [(speed x 0.1) + (grade x speed x 1.8) + 3.5] / 3.5, where fractional grade is expressed in decimal form and speed in meters per minute (1 mile per hour is equal to 26.8 meters per minute) [3].

Blood pressure (by sphygmomanometer) and a 12-lead electrocardiogram were recorded at rest, throughout exercise, and at regular intervals during recovery until heart rate and electrocardiogram had returned to baseline (recovery data not used for this study). Each subject was monitored throughout the test for ECG and heart rate by the Quinton 4500 stress system). Maximal heart rate was determined as the highest heart rate achieved during the last 30 seconds of exercise testing. Treadmill tests were terminated according to the guidelines recommended by the American College of Sports Medicine [3]. The same cardiologist, who was blinded to the genotype group, supervised all treadmill tests.

Echocardiographic evaluation

All subjects underwent a complete resting echocardiography examination using a Siemens-Accuson Sequoia machine (USA). Three consecutive cardiac cycles were analyzed and averaged for each patient. LVEF was measured using the modified biplane Simpson's method from the apical 4- and 2-chamber views. Transmitral inflow velocities were assessed by pulsed-wave Doppler, with the sample volume placed between the mitral leaflet tips in the apical 4-chamber view. The peak early (E) and late (A) transmitral velocities and the E-wave deceleration time (DT) of early filling velocity were assessed and the E/A ratio was calculated for the evaluation of diastolic function. One cardiologist performed all echocardiographic evaluations and was blinded to the genotype group.

Exercise Training

Patients exercised three times a week for 6 months. In the first month, each exercise session consisted of 10 min warm-up exercises, 15 min of aerobic exercise and 10 min of cool-down with stretching exercises. The aerobic exercise consisted of interval training - 5 sets of 3 min exercise at 70-75% of maximal heart rate interspersed with 1 min active recovery at 45-55% of maximal heart rate - performed on a treadmill or bicycle ergometer. In the following five months, aerobic exercise duration was progressively increased up to 35 minutes by increasing the number and duration of exercise sets (7 x 5 min), while maintaining the duration of active recovery. Heart rate was continuously monitored by ECG with each minute rhythm strip hard copy recorded. Absolute exercise intensity was adjusted progressively throughout the study

to ensure that all exercise-training sessions were performed within the established heart rate.

Genotyping

Genomic DNA was extracted from EDTA-treated whole blood according to a standard protocol. Genotyping of *ADRB1* Arg389Gly (rs1801253), *GNAS* -1211 G/A (rs6123837) and *GNAS* 2291 T/C (rs6026584) SNPs was performed by polymerase chain reaction (PCR). Specific genotyping conditions including primers, annealing temperature, restriction enzymes and product lengths for each polymorphism are described in Table 1. The digested products were electrophoresed in agarose gel (*GNAS* 2291 T/C and *GNAS* -1121: 2.5%, *ADRB1*: 3%). To ensure proper internal control, negative controls were used for each genotype analysis according to recent recommendations [10]. The restriction fragment length polymorphism (RFLP) data were assessed by two experienced and independent investigators who were blinded to the participants' data.

Table 1. Information on genotyping methods for each variant.

Gene	SNP	Gene location	Nucleotide variation	Primers	Annealing Temp	Restriction Enzyme	Product length
ADRB1	rs1801253	Exon	C/G	F - 5' CGCTCTGCTGGCTGCCCTTCTTCC 3' R - 5' TGGGCTTCGAGTTCACCTGCTATC 3'	60°C	Bcg I	G: 530 bp C: 342, 154, 34 bp
GNAS	rs6123837	5' UTR	G/A	F - 5' GTGGTGTTCCTGGTCTTCTCGGTGC 3' R - 5' CCCGAACACGAAGCCGCAGCC 3'	58°C	CviAII	G: 102 bp A: 35, 67 bp
GNAS	rs6026584	Intron	C/T	F - 5' TAAAGGCAGAATTATGCTGTTGGGA 3' R - 5' AGATCCGTGCCTCAGTTTCCAC 3'	58°C	SspI	T: 318, 190 bp C: 508 bp

SNP, single nucleotide polymorphism; UTR, untranslated region

Statistical Analysis

Normal data distribution was confirmed by the D'Agostino & Pearson omnibus test and Shapiro-Wilk normality test. Data are presented as mean ± SD, unless otherwise stated. Continuous variables with non-normal distribution were log-transformed before statistical testing and means were back-transformed for presentation. The chi-squared (χ^2) test was used to assess deviations of genotype distribution from the Hardy-Weinberg equilibrium (HWE). To evaluate differences between genotypes in

exercise tolerance and parameters of left ventricular function we used a one-way ANOVA test. To examine the main effect of exercise training on exercise tolerance and left ventricular function, a repeated measures general linear model was used. A two-way mixed model ANOVA was used to assess the effects of single nucleotide polymorphisms (SNP) on exercise training-mediated changes in exercise tolerance and left ventricular function (genotype x time). When significant interactions were observed, t-tests were applied to determine the location of differences within each genotype relative to baseline as well as between genotypes at baseline and follow-up. To account for the inflation of type I error that may occur with multiple comparisons, statistical significance was corrected with Holm-Bonferroni method where appropriate. When all groups (genotypes) manifested a significant change in exercise tolerance or left ventricular function, the group effect was analyzed by independent samples ANOVA with change scores entered as dependent variables. The change scores (absolute differences) were calculated as the values recorded after exercise training (follow-up) minus the values recorded at baseline. Genotype x time interactions were adjusted for potential confounders, such as age, gender, BMI, hypertension, diabetes, smoking status and medications. $P < 0.05$ was considered indicative of statistical significance.

Results

From the 67 patients who were eligible to participate in this study, 1 was unable to attend the exercise programme and 1 did not perform the follow-up echocardiographic evaluation. Moreover, we failed to extract enough DNA from the blood samples of 4 patients. Therefore, a total of 61 patients were included in the analysis. No adverse events were registered during exercise testing or during aerobic interval training. Our patients tolerated the exercise training protocol and did not have any problems in maintaining their target heart rate range throughout the exercise sessions.

Baseline characteristics

Patient baseline characteristics are shown in Table 2. There were no significant differences between all genotypes for age, gender, anthropometric characteristics and cardiovascular risk factors (data not shown).

In the *GNAS* 2291 T/C variant, the proportion of patients taking statins was lower in 2291T homozygous compared with the other genotypes (50% vs. 93% vs. 75%, χ^2 = 6.38, df = 2, *P*=0.041). Likewise, the proportion of patients taking angiotensin-II antagonists was higher in individuals homozygous for the *GNAS* 1211G allele than in those heterozygous and homozygous for the 1211A variant (40% vs. 9% vs. 8%, χ^2 = 7.89, df = 2, *P*=0.019). No more associations were found between patients' genotypes and medications.

The allele and genotype frequencies of the *ADRB1* Arg389Gly and *GNAS* -1211 G/A, *GNAS* 2291 T/C variants are shown in Figure 1. There were no deviations from the Hardy – Weinberg equilibrium expectations in the *ADRB1* (χ^2 = 0.01, df=2, *P*=0.92), *GNAS* -1211 (χ^2 = 0.19, df=2, *P*=0.66) and *GNAS* 2291 (χ^2 = 0.58, df=2, *P*=0.75) SNPs (χ^2 = 0.06, df=2, *P*=0.97).

Table 2. Demographic and clinical characteristics of patients at baseline

	N=61
Age (yrs)	64.9 ± 11.1
Sex (M/F)	43/18
BMI (kg/m^2)	28.5 ± 4.4
LVEF (%)	48.2 ± 8.9
NYHA	
I	3 (0.05)
II	28 (0.46)
III	30 (0.49)
MI	32 (0.52)
Valvular disease	3 (0.05)
Hypertension	49 (0.77)
Diabetes Mellitus	20 (0.33)
Beta-blockers	48 (0.79)
Alpha-adrenergic antagonists	6 (0.10)
ACE inhibitors	22 (0.36)
AT1R antagonists	12 (0.20)
Calcium channel blockers	14 (0.23)
Diuretics	12 (0.20)
Statins	50(0.82)
Spironolactone	5 (0.08)

ACE, Angiotensin converting enzyme; AT1R, angiotensin-II type 1 receptors; BMI; Body mass index; MI, myocardial infarction; NYHA, New York heart association. Data is presented as mean ± SD or absolute frequency (percentage).

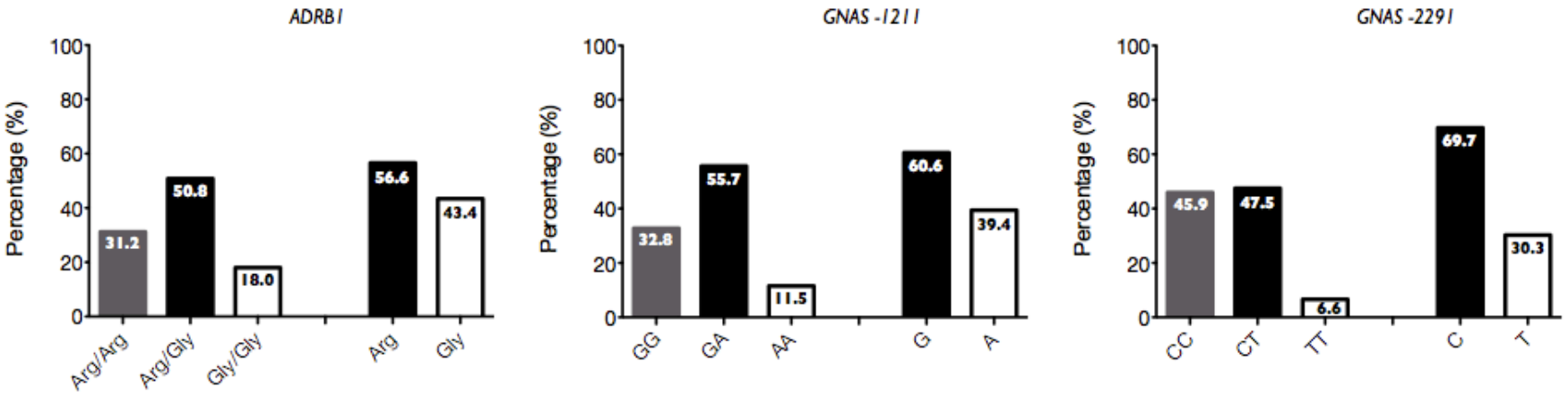

Figure 1. Genotype and allele frequencies of the three variants tested

Left ventricular ejection fraction

At baseline, there were no differences between genotypes in LVEF (Table 3). Exercise training increased the average LVEF (baseline: 47.8 ± 8.9% vs. follow-up: 50.8 ± 8.2%, $P<0.001$). This response did not differ between genotypes (Table 3).

Left ventricular diastolic function

Mitral inflow velocities could not be assessed in three patients that suffered from atrial fibrillation. At baseline, there were no differences in the E/A ratio between genotypes (Table 3). The average E/A ratio increased after exercise training (baseline: 0.92 ± 0.23 vs. follow-up: 0.99 ± 0.16, $P < 0.01$). This response was similar between genotypes (Table 3).

There were no differences between genotypes in the deceleration time at baseline, apart from those related with the *GNAS* -1211 variant ($F = 3.99$, $P = 0.024$). The deceleration time at baseline was lower in patients homozygous for the -1211A allele compared with the other genotypes (Table 3). The average deceleration time decreased after exercise training (baseline: 235.1 ± 46.1 ms vs. follow-up: 220.5 ± 24.1 ms, $P = 0.002$). However, exercise training induced different deceleration time responses between *GNAS* -1211 genotypes (genotype x time interaction: $F = 3.34$, $P = 0.043$). The deceleration time decreased significantly after exercise training in patients heterozygous ($P = 0.006$) and homozygous for the -1211G allele ($P = 0.018$), while it remained unchanged in patients homozygous for the -1211A allele. These results remained significant after adjustment for patients' demographic characteristics, cardiovascular risk factors, medications and different subsets of heart failure. There were no differences between all the other genotypes in the deceleration time responses to exercise training.

Exercise tolerance

Exercise tolerance at baseline was comparable among all genotypes (Table 3). There was a main effect of exercise training on exercise tolerance (baseline: 4.01 ± 1.18 vs. follow-up: 4.52 ± 1.33 METs, $P < 0.001$). Exercise training induced different exercise tolerance responses among *ADRB1* genotypes (genotype x time interaction: $F = 3.29$, $P = 0.044$). All *ADRB1* genotypes increased exercise tolerance over time (Table 3), but comparisons of absolute changes over the 6 months (i.e. absolute difference from

baseline) confirmed that it increased more in patients homozygous for the 389Gly allele than in those who were 389Arg homozygotes (0.88 ± 0.80 vs. 0.33 ± 0.50 METs, $P = 0.039$). These results remained significant when the effects of age, gender, cardiovascular risk factors, medications (including all beta-blockers subtypes pooled together) and different subsets of heart failure were taken into consideration. In addition, we made an adjustment for Metoprolol and Atenolol because recent evidence has suggested that they may reduce inotropic reserve in 389Arg homozygotes with little impact on 389Gly allele carriers [24]. Indeed, differences between genotypes for the training response were no longer different when Metoprolol and Atenolol were taken into consideration. No differences between all the other genotypes were observed in the exercise tolerance responses to exercise training.

Table 3. Baseline and follow-up values of left ventricular function and exercise tolerance among *ADRB1, GNAS* -1211 and *GNAS* 2291 variants

		LVEF (%)		E/A ratio		DT		METs	
		Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
ADRB1	GG	46.9 ± 13.7	50.4 ± 14.2	0.97 ± 0.26	0.98 ± 0.15	233.4 ± 65.1	226.5 ± 37.2	3.9 ± 1.2	4.8 ± 1.5 ** †
	RG	47.9 ± 9.8	50.6 ± 9.2	0.94 ± 0.21	1.01 ± 0.16	230.1 ± 46.8	218.3 ± 23.8	4.1 ± 1.2	4.6 ± 1.3 **
	RR	49.8 ± 9.6	52.3 ± 7.5	0.88 ± 0.14	0.98 ± 0.16	242.1 ± 24.3	225.3 ± 13.7	4.1 ± 1.2	4.4 ± 1.3 **
GNAS -1211	GG	49.6 ± 5.8	52.7 ± 5.7	0.90 ± 0.16	0.98 ± 0.13	238.6 ± 40.3	224.3 ± 21.8 **	4.1 ± 1.1	4.6 ± 1.2
	GA	48.4 ± 12.0	50.9 ± 10.8	0.91 ± 0.19	0.98 ± 0.14	239.9 ± 40.6	224.6 ± 22.2 **	3.9 ± 1.2	4.4 ± 1.3
	AA	44.2 ± 11.9	47.3 ± 12.2	1.10 ±0.30	1.12 ± 0.25	196.3 ± 51.0 *	201.4 ± 30.4	4.5 ± 1.6	5.1 ± 1.8
GNAS 2291	CC	47.8 ± 5.3	52.1 ± 7.2	0.96 ± 0.21	1.02 ± 0.16	230.7 ± 47.8	220.7 ± 24.6	4.2 ± 1.2	4.7 ± 1.4
	CT	49.1 ± 11.6	51.1 ± 10.7	0.88 ± 0.17	0.96 ± 0.15	238.9 ± 41.6	222.6 ± 24.2	3.9 ± 1.2	4.4 ± 1.3
	TT	47.7 ± 10.2	50.9 ± 9.4	1.01 ± 0.32	0.99 ± 0.09	230.4 ± 68.8	223.7 ± 32.4	3.9 ± 1.4	4.9 ± 1.5

DT, deceleration of early mitral flow; LVEF, left ventricular ejection fraction; MET, metabolic equivalents. * Significantly lower than GA and GG genotypes. $P<0.05$; ** Significantly different than baseline. $P<0.05$; † Significantly higher than Arg/Arg genotype. $P<0.05$.

Hemodynamics

Resting hemodynamics did not differ between any of the genotypes (Table 4). There was a main effect of exercise training in resting heart rate (baseline: 71.1 ± 14.0 bpm vs. follow-up: 67.8 ± 9.9 bpm, $P<0.0001$), peak heart rate (baseline: 122.3 ± 20.2 bpm vs. follow-up: 126.6 ± 15.8 bmp, $P=0.001$), resting diastolic blood pressure (baseline: 78.2 ± 5.3 mmHg vs. follow-up: 76.5 ± 4.7 mmHg, $P=0.014$), resting systolic blood pressure (baseline: 123.1 ± 12.0 mmHg vs. follow-up: 125.4 ± 11.8 mmHg, $P=0.037$) and peak systolic blood pressure (baseline: 155.5 ± 15.3 mmHg vs. follow-up: 158.5 ± 12.2 mmHg, $P=0.030$). No changes over time were found in peak diastolic blood pressure (baseline: 77.9 ± 5.2 mmHg vs. follow-up: 78.5 ± 4.7 mmHg, $P=0.014$). Furthermore, there were no genotype effects on resting and peak hemodynamic responses to exercise training.

Table 4. Baseline and follow-up values of resting and exercise hemodynamics among *ADRB1, GNAS* -1211 and *GNAS* 2291 variants

		rHR (bpm)		rSBP (mmHg)		rDBP (mmHg)		pHR (bpm)		pSBP		pDBP	
		Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
ADRB1	GG	68.0±14.1	65.9±7.9	120.0±10.9	122.5±10.6	78.2±6.4	75.0 ± 5.5	123.7±14.3	126.7±12.2	151.4±14.8	155.5±12.7	78.2±6.0	77.5±4.2
	RG	70.1±15.8	66.9±10.5	126.5±12.4	127.1±14.3	79.4±5.3	76.9 ± 4.9	121.1±22.6	127.7±17.0	154.8±15.3	158.4±11.8	78.7±4.5	78.8±4.9
	RR	74.5±13.4	69.4±10.1	120.5±12.6	124.6±8.4	76.6±5.5	76.7 ± 3.9	120.5±12.6	123.9±16.4	156.1±16.7	159.8±13.4	76.1±5.9	78.3±4.7
GNAS -1211	GG	66.4±14.1	64.3±10.4	125.8±11.9	127.1±14.2	77.3±5.3	76.4 ± 5.6	116.1±20.3	120.9±16.0	156.5±13.1	158.7±12.7	77.3±4.1	77.7±4.4
	GA	73.8±14.0	69.2±9.7	123.2±13.5	125.3±11.3	78.7±6.1	76.7 ± 4.0	121.7±19.2	125.8±14.7	152.3±17.5	156.9±12.6	77.6±5.9	78.9±4.9
	AA	71.7±13.0	68.9±7.8	117.9±5.7	121.0±7.6	79.3±4.5	75.7 ± 6.1	134.6±20.3	142.6±10.1	160.0±10.8	163.1±10.8	80.0±5.8	77.1±4.9
GNAS 2291	CC	69.7±12.0	67.0±9.2	120.4±10.4	121.2±8.7	77.5±5.4	75.6 ± 4.6	123.7±21.8	127.4±16.9	156.4±13.8	159.6±13.2	77.5±5.7	78.1±4.2
	CT	72.7±16.3	68.3±10.7	127.1±13.8	130.1±13.7	79.7±5.7	77.6 ± 4.8	118.8±19.6	124.3±15.6	152.7±17.5	156.5±12.1	78.2±5.1	78.8±5.1
	TT	69.7±12.6	66.0±11.1	118.8±8.5	121.7±5.8	73.8±4.8	75.0 ± 5.0	122.5±11.4	133.0±3.5	155.0±13.5	161.7±2.9	76.3±4.8	76.7±7.6

pHR; peak heart rate; pSBP; peak systolic blood pressure; pDBP; peak diastolic blood pressure; rHR, resting heart rate; rSBP, resting systolic blood pressure; rDBP, resting diastolic blood pressure

Discussion

The main findings of this study were: (1) *GNAS* -1211G allele carriers had elevated deceleration time of early mitral flow compared to -1211A allele homozygotes, (2) exercise training decreased the deceleration time in *GNAS* -1211G allele carriers, but not in -1211A allele homozygotes, (3) *ADRB1* 389Gly homozygotes had a greater training-induced increase in exercise tolerance than 389Arg homozygotes. Nevertheless, Metoprolol and Atenolol abolished these differences between *ADRB1* genotypes.

The current study presents a novel association between *GNAS* -1211 A/G polymorphism and diastolic dysfunction. In particular, the deceleration time of early mitral flow was higher in -1211G allele carriers compared to -1211A homozygotes. The -1211G-A allele transition has been shown to result in altered transcription factor binding and promoter activity [19]. Therefore, it is possible that in heart failure, which results in Gαs proteins uncoupling, the -1211G allele may contribute to depression in intracellular adrenergic signalling. Consequently, calcium regulation may be impaired and contribute to diastolic dysfunction [25]. On the other hand, exercise training has been shown to improve adrenergic responsiveness and diastolic dysfunction in heart failure patients [11, 41]. Thus, it may be argued that exercise training may restore adrenergic responsiveness in -1211G allele carriers, and consequently reduce their deceleration time.

We found no association between *ADRB1* and *GNAS* polymorphisms and LVEF in heart failure patients. These findings are in agreement with those reported in patients with ischemic and idiopathic cardiomyopathies [40]. Wagoner *et al* also found no association between *ADRB1* gene variants, including the one studied here, and baseline LVEF [40]. These observations have been corroborated in large clinical trials with heart failure patients [14, 31]. On the other hand, this association was not tested for common variants within the *GNAS* gene [18]. These data suggest that *ADRB1* and *GNAS* polymorphisms are probably no candidates to influence left ventricular systolic function in heart failure patients.

The impact of these common variants on the left ventricular response to exercise training remains unclear in heart failure patients. The present investigation confirmed

that exercise training increases LVEF in heart failure patients [20, 22]. However, this training response was not associated with *ADRB1* and *GNAS* polymorphisms. These observations are in agreement with previous clinical trials that tested the association between *ADRB1* gene variants and the left ventricular response to pharmacological intervention [14, 31]. The reasons for this lack of association remain unclear. However, a number of potential explanations are proposed. LVEF is considered to be a more accurate measure of ventricular – arterial coupling than contractility alone [4]. Thus, the absence of changes in LVEF may not correspond to the absence of changes in cardiac contractility [26]. This has been supported in some studies where the association of Arg389Gly polymorphism with more accurate measures of cardiac contractility did not correspond to an association with LVEF [31]. In addition, we measured systolic function at rest, while these variants exert their maximal influence under extreme conditions that stimulate the release of catecholamines, such as exercise conditions [30, 32]. Thus, future studies should test the association between *ADRB1* and *GNAS* polymorphisms and left ventricular function measured during exercise conditions.

ADRB1 389Gly homozygotes had a greater improvement in exercise tolerance than 389Arg homozygotes. This observation was somewhat unanticipated. While the 389Gly allele is associated with lower agonist-mediated adenylate cyclase and inotropic reserve [31, 32] the 389Arg allele is more susceptible to desensitization when the receptor is exposed to increased concentrations of catecholamines [33]. Thus, receptor desensitization may impair the ability of exercise to increase inotropic reserve and exercise tolerance in 389Arg allele homozygotes. However, differences between ADRB1 genotypes were abolished when they were adjusted for metoprolol and atenolol. In a recent study, dobutamine-induced increase in contractility was abolished with pretreatment with metoprolol in 389Arg homozygotes [24]. Conversely, metoprolol had little effect on 389Gly allele carriers [24]. These data suggest that metoprolol might have prevented exercise training from improving inotropic reserve and exercise tolerance. On the other hand, metoprolol has been associated with an increase in cardiac β-adrenergic receptor density [21], which might be beneficial for 389Arg allele carriers. Thus, future studies with different cohorts of heart failure patients are encouraged to evaluate the association between *ADRB1*

genotypes and exercise tolerance at baseline and following endurance training with and without metoprolol.

In this study, *ADRB1* and *GNAS* polymorphisms were not associated with baseline or training-induced changes in resting or peak heart rate and blood pressures. These results are comparable with those observed in healthy subjects [9, 43] as well as heart failure patients [40]. As of now, little information exists regarding association of genetic variants with heart rate and/or blood pressure responses to exercise training [39]. However, the results of the current study suggest that *ADRB1* and *GNAS* polymorphisms do not play a major role in resting or exercise heart rate and blood pressures.

This study has some limitations, one of which is its small sample size. However, it has been previously emphasized that recruiting large samples of subjects, not to mention heart failure patients, to long-term exercise training programs is rather difficult [5]. This has been illustrated by many studies, which tested the interventional response of individuals with different adrenergic genotypes [8, 9, 30]. In the present study we included patients with HFREF and HFPEF. Similar to other reports [16, 28, 41], we have shown that exercise training can improve diastolic dysfunction and exercise tolerance in both subsets of heart failure [2]. In conclusion, this study shows that exercise training attenuates diastolic dysfunction in *GNAS* -1211G allele carriers to levels close to those of -1211A homozygotes. In addition, our data shows that the *ADRB1* Arg389Gly polymorphism influences the exercise tolerance response to training but not left ventricular function at baseline and after chronic exercise in heart failure patients.

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Study IV

Genetic variation in the PPARGC1A signalling cascade modulates cardiac function and exercise tolerance responses to exercise training in heart failure patients

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Submitted

Abstract

Background: Abnormal cardiac energy metabolism is a common feature of heart failure (HF). This is associated with the reduced expression of peroxisome proliferator-activated receptor (PPAR) γ coactivator-1α (PPARGC1A), PPARs and nuclear respiratory factors (NRF).

Aims: The aim of the present study was to investigate whether variants in the *PPARGC1A* (rs8192678, rs6821591), *PPARA* (rs1800206, rs4253778), *PPARD* (rs2016520) and *NRF2* (rs12594956) genes are associated with left ventricular function and exercise tolerance at baseline or after exercise training in HF patients.

Methods: A total of 61 patients completed a 6-month aerobic exercise-training program. Left ventricular ejection fraction (LVEF), mitral inflow velocities and exercise tolerance (METs) were assessed at baseline and after exercise training. Gene variants were detected with restriction fragment length polymorphism analysis.

Results: There were no differences between genotypes in mitral inflow velocities. *PPARGC1A* Ser482 allele homozygotes had a lower LVEF at baseline compared with heterozygotes (P=0.027). Exercise training increased LVEF more pronouncedly in *PPARGC1A* Ser482 allele homozygotes compared with heterozygotes (P=0.037) but not compared with Gly482 allele homozygotes (P=0.170). In addition, the exercise training-induced increase in exercise tolerance was more pronounced in *PPARA* 162Val carriers than in 162Leu homozygotes (P=0.032). Likewise, exercise training increased exercise tolerance in *NRF2* A allele carriers (P<0.005), but not in G allele homozygotes.

Conclusions: This study suggests that aerobic interval training improves systolic dysfunction in HF patients with the 'unfavourable' *PPARGC1A* Ser482Ser variant. Our data also suggest that *PPARA* Leu162Val and *NRF2* A/C variants are associated with the training response of exercise tolerance in HF patients.

Key words: Aerobic exercise, single nucleotide polymorphism, metabolism, fatty acid, PGC-1α

Introduction

Abnormal cardiac energy metabolism is a well-known mechanism that contributes to heart failure. Numerous components of the cardiac metabolic machinery are affected in heart failure, which include, but are not limited to, changes in substrate uptake and oxidation as well as in oxidative phosphorylation (40, 54).

The peroxisome proliferator-activated receptor γ coactivator-1α (PPARGC1A) controls cardiac metabolism through the co-activation of multiple transcription factors, including the peroxisome proliferator-activated receptors (PPARs) (16, 37). The PPARGC1A also stimulates mitochondrial biogenesis through the expression of nuclear respiratory factors 1 (NRF1) and 2 (NRF2) (50). Reduced expression of *PPARGC1A* and its downstream transcription factors has been documented in mice and human heart failure in association with reduced fatty acid oxidation and left ventricular ejection fraction (LVEF) (47, 49). Common variants in the *PPARGC1A* and *PPAR* genes have been associated with metabolic disorders and heart failure (4, 7). However, the association between common variations in genes that are involved in cardiac metabolism and cardiac function has never been investigated in heart failure patients.

Exercise intolerance is the cardinal manifestation of heart failure. Exercise training has been shown to improve exercise tolerance but also left ventricular function in patients with heart failure (25, 42). There is evidence from healthy subjects and animal studies to support that exercise training improves mitochondrial oxidative metabolism and increases the expression of proteins involved in the PPARGC1A signalling cascade in heart and skeletal muscles (6, 51). This has been confirmed in animal studies of heart failure (33, 55). Moreover, associations between genetic variations in the *PPARGC1A*, *PPAR* and *NFR2* genes and endurance performance have been documented in athletes and normal individuals (14, 15, 26, 38). However, the role that common gene variations involved in muscle metabolism and mitochondrial biogenesis have on exercise tolerance has never been investigated in heart failure patients. Identification of 'cardioprotective' genotypes in response to endurance training, i.e. allowing for the attainment of the greatest possible gains is of potential clinical relevance and applicability for cardiac patients. Training at relatively high intensities (i.e. moderate-to-intense training) is also necessary to maximize gains in myocardial function and

exercise tolerance (58). Thus, the aim of this study was to examine whether *PPARGC1A* (rs8192678, rs6821591), *PPARA* (rs1800206, rs4253778), *PPARD* (rs2016520) and *NRF2* (rs12594956) genes variants are associated with left ventricular function and exercise tolerance in heart failure patients that were involved in an endurance-training program.

Methods

Subjects

Sixty-seven heart failure patients in NYHA (New York Heart Association) class I-III with either preserved or reduced left ventricular ejection fraction (HFPEF & HFREF) were included in the study as previously described (1). Exclusion criteria included uncontrolled hypertension, unstable angina pectoris, uncontrolled cardiac arrhythmias, significant ischemic electrocardiogram ST-T changes during the initial stages of the exercise tolerance test (Modified Bruce protocol), uncontrolled metabolic disease (e.g. uncontrolled diabetes and thyroid disease) and medical conditions that limit participation in exercise (e.g. peripheral arterial occlusive disease and musculoskeletal disorders). Patients who completed less than 80% of prescribed exercise sessions were excluded from the analysis. The hospital ethics committee approved the study protocol and written informed consent was obtained from every patient. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki.

Exercise Testing

Each subject performed a symptom limited, graded exercise treadmill test according to a standard Modified Bruce protocol. To examine exercise tolerance, treadmill exercise time was recorded and metabolic equivalents (METs) estimated according to the guidelines provided by the American College of Sports Medicine:

MET = [(speed x 0.1) + (grade x speed x 1.8) + 3.5] / 3.5, where fractional grade is expressed in decimal form and speed in meters per minute (1 mile per hour is equal to 26.8 meters per minute) (2).

Blood pressure (by sphygmomanometer) and a 12-lead electrocardiogram were recorded at rest, throughout exercise, and at regular intervals during recovery until heart rate and electrocardiogram had returned to baseline. Each subject was monitored throughout the test for ECG and heart rate by the Quinton 4500 stress system. Maximal heart rate was determined as the highest heart rate achieved during the last 30 seconds of exercise testing. Treadmill tests were terminated according to the guidelines recommended by the American College of Sports Medicine (2). The same cardiologist, who was blinded to the genotype group, supervised all treadmill tests (22).

Echocardiographic evaluation

All subjects underwent a complete resting echocardiography examination using a Siemens-Accuson Sequoia machine (USA). Three consecutive cardiac cycles were analyzed and averaged for each patient. LVEF was measured using the modified biplane Simpson's method from the apical 4- and 2-chamber views. Transmitral inflow velocities were assessed by pulsed-wave Doppler, with the sample volume placed between the mitral leaflet tips in the apical 4-chamber view. The peak early (E) and late (A) transmitral velocities and the E-wave deceleration time (DT) of early filling velocity were assessed and the E/A ratio was calculated for the evaluation of diastolic function. An experienced cardiologist performed all echocardiographic evaluations and was blinded to the genotype group (21).

Exercise Training

Patients exercised three times a week for 6 months. In the first month, each exercise session consisted of 10 min warm-up exercises, 15 min of aerobic exercise and 10 min of cool-down with stretching exercises. The aerobic exercise consisted of interval training - 5 sets of 3 min exercise at 70-75% of maximal heart rate interspersed with 1 min active recovery at 45-55% of maximal heart rate - performed on a treadmill or bicycle ergometer. In the following five months, aerobic exercise duration was progressively increased up to 35 minutes by increasing the number and duration of exercise sets (7 x 5 min), while maintaining the duration of active recovery. Heart rate was continuously monitored by ECG with each minute rhythm strip hard copy recorded. Absolute exercise intensity was adjusted progressively throughout the study

to ensure that all exercise-training sessions were performed within the established heart rate.

Genotyping

Genomic DNA was extracted from EDTA-treated whole blood according to a standard protocol. Genotyping of the single nucleotide polymorphisms (SNPs) *PPARGC1A* Gly482Ser (rs8192678), *PPARGC1A* 2962 G/A (rs6821591), *PPARA* Leu162Val (rs1800206), *PPARA* Intron 7 C/G (rs4253778), *PPARD* 294 C/T (rs2016520) and *NRF2* A/C (rs12594956) was performed with polymerase chain reaction (PCR) as described in previous studies (13, 14). Information on specific genotyping conditions including primers, PCR annealing temperature, restriction enzymes and product lengths obtained for each allele is shown in Table 1. The digested products were electrophoresed in a 3% agarose gel. To ensure proper internal control negative controls were used for each genotype analysis according to recent recommendations (10). Two experienced and independent investigators who were blind to the participants' data assessed the restriction fragment length polymorphism (RFLP) results.

Table 1. Description of genotyped variants

SNP	RefSNP ID	Gene location	SNP	Primers	Annealing Temp	Restriction Enzyme	Product length
PPARGC1A Gly482Ser	rs8192678	Exon	G/A	F - 5' TAAAGATGTCTCCTCTGATT 3' R - 5' GGAGACACATTGAACAATGAATAGGATTG 3'	50°C	HpaII	G: 209, 169 bp A: 378 bp
PPARGC1A 2962 G/A	rs6821591	3' UTR	G/A	F - 5' CAATAACAACAATGGTTTACATGA 3' R - 5' CGAACATTTTGAAGTTCTAGGTTTTACG 3'	50°C	Mlu 1	G: 280, 30 bp A: 310 bp
PPARA Leu162Val	rs1800206	Exon	C/G	F - 5' GACTCAAGCTGGTGTATGACAAGT 3' R - 5' CGTTGTGTGACATCCCGACAGAAT 3'	63°C	Hinf I	C: 117 bp G: 93, 24 bp
PPARA Intron 7 C/G	rs4253778	Intron	C/G	F - 5' ACAATCACTCCTTAAATATGGTGG 3' R - 5' AAGTAGGGACAGACAGGACCAGTA 3'	59°C	Taq I	C: 266 bp G: 216, 50 bp
PPARD 294 C/T	rs2016520	5' UTR	C/T	F - 5' GAAGGAGCAGGAGCAGAAGA 3' R - 5' CAGTCATAGCTCTGGCATCG 3'	59°C	Bsl1	T: 187 C: 141, 46 bp
NRF2 A/C	rs12594956	Intron	A/C	F - 5' TAAAATGAATAAAGGTGGGGGT 3' R - 5' TAAGAGTGGAAGGGTGGAGAA 3'	50°C	Mfe I	C: 407 bp A: 277, 130 bp

SNP, single nucleotide polymorphism; UTR, untranslated region

Statistical Analysis

Normal data distribution was confirmed by the D'Agostino & Pearson omnibus test and the Shapiro-Wilk normality test. Data are presented as mean ± SD, unless otherwise stated. Continuous variables with non-normal distribution were log-transformed before statistical testing and means were back-transformed for presentation. The chi-squared (χ^2) test was used to assess deviations of genotype distribution from the Hardy-Weinberg equilibrium (HWE). To evaluate differences between genotypes in exercise tolerance and parameters of left ventricular function at baseline one-way ANOVA test was used. To examine the effect of exercise training on average exercise tolerance and left ventricular function for all patients (main effect), a repeated measures general linear model was used. A two-way (genotype x time) mixed model ANOVA was used to assess the effects of gene variants on exercise training-mediated changes in exercise tolerance and left ventricular function. When significant interactions were observed, *t*-tests were applied to determine the location of differences within each genotype relative to baseline as well as between genotype at baseline and follow-up. To account for the inflation of type I error that may occur with pairwise multiple comparisons, statistical significance was corrected with Holm-Bonferroni method where appropriate. When all groups (genotypes) manifested a significant change in exercise tolerance or left ventricular function, the group effect was analyzed by independent samples ANOVA with *change scores* (absolute differences) entered as dependent variables. The *change scores* were calculated as the values recorded after exercise training (follow-up) minus the values recorded at baseline. Genotype x time interaction was then adjusted for potential confounders, such as age, gender, BMI, hypertension, diabetes, smoking status, medications and different subsets of heart failure. $P < 0.05$ was considered indicative of statistical significance.

Results

From the 67 patients who were eligible to participate in this study, 1 was unable to attend the exercise programme and 1 did not perform the follow-up echocardiographic evaluation. Moreover, we failed to extract enough DNA from the blood samples of 4 patients. Therefore, a total of 61 patients were included in the following analysis. No adverse events were registered during exercise testing or during aerobic interval training. Our patients tolerated the exercise training protocol and did not have any problems in maintaining their target heart rate range throughout the exercise sessions.

Table 2. Demographic and clinical characteristics of patients at baseline

	N=61
Age (yrs)	64.9 ± 11.1
Sex (M/F)	43/18
BMI (kg/m^2)	28.5 ± 4.4
LVEF (%)	48.2 ± 8.9
NYHA	
I	3 (5%)
II	28 (46%)
III	30 (49%)
MI	32 (52%)
Valvular disease	3 (5%)
Hypertension	49 (77%)
Diabetes Mellitus	20 (33%)
Beta-blockers	48 (79%)
Alpha-adrenergic antagonists	6 (10%)
ACE inhibitors	22 (36%)
AT1R antagonists	12 (20%)
Calcium channel blockers	14 (23%)
Diuretics	12 (20%)
Statins	50(82%)
Spironolactone	5 (8%)

ACE, Angiotensin converting enzyme; AT1R, angiotensin-II type 1 receptors; BMI, Body mass index; LVEF, Left ventricular ejection fraction; MI, myocardial infarction; NYHA, New York heart association. Data is presented as mean ± SD or absolute frequency (percentage).

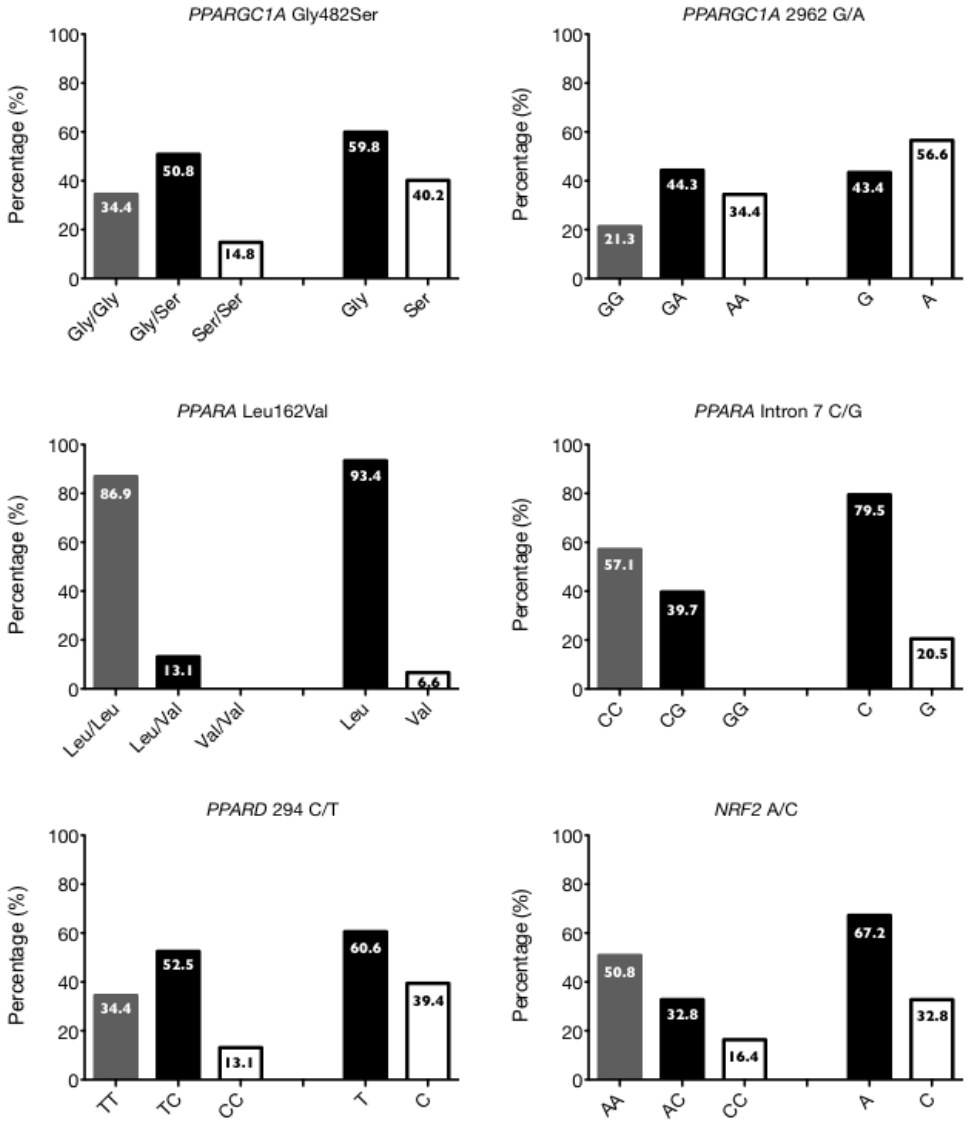

Figure 1. Genotype and allele absolute and relative (%) frequencies.

Baseline characteristics

Baseline characteristics from all patients are shown in Table 2. The proportion of patients with the *NRF2* CC genotype taking statins was higher than those carrying the *NRF2* CA and AA genotypes (100 % vs. 95% vs. 67%, χ^2 = 9.05, df=2, *P*=0.01). There were relatively more patients with the *NRF2* CC genotype taking calcium channel blockers compared to those with the *NRF2* CA and AA genotypes (60 % vs. 15% vs. 16%, χ^2 = 9.57, df=2, *P*<0.01). No differences were found between genotypes for age, gender, and anthropometric characteristics.

The allele and genotype frequencies of the *PPARGC1* Gly482Ser, *PPARGC1* 2962 G/A, *PPARA* Leu162Val, *PPARA* Intron 7 C/G, *PPARD* 294 C/T and *NRF2* A/C variants are shown in Figure 1. There were no deviations from the HWE in any the variants we studied, i.e. *PPARGC1A* Gly482Ser (χ^2 = 0.14, df=2, *P*=0.93), *PPARGC1A* 2962 G/A (χ^2 = 0.30, df=2, *P*=0.86), *PPARA* Leu162Val (χ^2 = 0.00, df=2, *P*=1.00), *PPARA* Intron 7 C/G (χ^2 = 2.68, df=2, *P*=0.26), *PPARD* 294 C/T (χ^2 = 0.39, df=2, *P*=0.82) and *NRF2* A/C SNPs (χ^2 = 1.85, df=2, *P*=0.40).

Left ventricular ejection fraction

Values of left ventricular function parameters and exercise tolerance at baseline and following exercise training (follow-up) are provided in Table 3. At baseline, there were significant differences between *PPARGC1A* Gly482Ser genotypes in LVEF (*F* = 3.68, *P*=0.031, Table 3). *PPARGC1A* Ser482 allele homozygotes presented lower baseline LVEF than heterozygotes (F = 3.41, P=0.027) and values also tended to be lower than Gly482 allele homozygotes (P=0.059). In addition, the response to exercise training varied between *PPARGC1A* Gly482Ser genotypes (genotype x time interaction: *F* = 3.34, *P*=0.043). All genotypes increased LVEF after exercise training (Table 3), but comparisons of absolute differences over time (i.e. difference from baseline to follow-up) showed that it increased more in Ser482 allele homozygotes than in heterozygotes (3.9 ± 3.1% vs. 2.3 ± 2.1 %, *P*=0.037), but not when compared with 482Gly homozygotes (3.9 ± 3.1% vs. 2.9 ± 2.3 %, *P*=0.170). These differences remained significant after adjustment for demographic and anthropometric characteristics, cardiovascular risk factors, medications and different subsets of heart failure.

Left ventricular diastolic function

Mitral inflow velocities could not be assessed in three patients with atrial fibrillation. No differences were found between genotypes for the E/A ratio at baseline (Table 3). Exercise training increased the average E/A ratio (baseline: 0.92 ± 0.23 vs. follow-up: 0.99 ± 0.16, $P < 0.01$), but the E/A ratio response to exercise training did not differ between genotypes. Furthermore, there were no differences between genotypes in the deceleration time at baseline (Table 3). The average deceleration time decreased after exercise training (baseline: 235.1 ± 46.1 ms vs. follow-up: 220.5 ± 24.1 ms, $P = 0.002$), but its response to exercise training was similar between genotypes.

Exercise tolerance

Exercise tolerance at baseline was comparable across genotypes (Table 3). Exercise training increased the average exercise tolerance (baseline: 4.01 ± 1.18 vs. follow-up: 4.52 ± 1.33 METs, $P < 0.001$). Exercise training induced different exercise tolerance responses between *PPARA* Leu162Val genotypes (genotype x time interaction: $F = 4.88$, $P=0.031$). All genotypes increased the exercise tolerance after exercise training ($P < 0.05$), but analysis of absolute differences over time indicates that the exercise tolerance increased more in 162Val carriers than in 162Leu homozygous individuals (0.88 ± 0.81 METs vs. 0.41 ± 0.51 METs, $P = 0.032$). Exercise tolerance responses also differed between *NRF2* A/C genotypes (genotype x time interaction: $F = 3.2$, $P=0.047$). Exercise tolerance increased in patients with the *NRF2* AA and AC genotype ($P < 0.001$), while it remained unchanged in patients with the *NRF2* CC genotype. Differences between *NRF2* genotypes were of borderline significance after correction for statins (genotype x time: $F = 2.9$, $P=0.058$) and calcium channel blockers (genotype x time: $F = 2.8$, $P=0.068$), but remained significant after correction for the other potential confounders. Subsequent analysis revealed that exercise tolerance still increased after exercise training in patients with the *NRF2* AA genotype ($P<0.001$), but it remained unchanged in heterozygotes and G allele homozygotes after correction for statins. On the other hand, when differences were corrected for calcium channel blockers, exercise tolerance improved in the A allele carriers ($P<0.001$), while it remained unaltered in patients with the CC genotype. No more differences were found between genotypes for exercise tolerance responses to exercise training.

Table 3. Baseline and follow-up values of left ventricular function and exercise tolerance among *PPARGC1A* Gly482Ser, *PPARGC1A* 2962 G/A, *PPARA* Leu162Val, *PPARA* Intron C/G, *PPARD* 294 C/T, *NRF2* A/C variants

		LVEF (%)		E/A ratio		DT		METs	
		Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
PPARGC1A G482S	GG	48.0 ± 10.0	50.9 ± 10.3 **	0.92 ± 0.17	0.97 ± 0.14	234.1 ± 40.4	222.1 ± 24.1	4.1 ± 1.2	4.4 ± 1.3
	GS	50.2 ± 9.2	52.5 ± 8.2 **	0.91 ± 0.19	1.00 ± 0.14	237.4 ± 43.2	223.6 ± 19.7	4.1 ± 1.2	4.6 ± 1.3
	SS	42.7 ± 12.8 *	46.5 ± 11.9 **	1.01 ± 0.29	1.03 ± 0.23	224.8 ± 64.9	215.1 ± 36.7	3.9 ± 1.1	4.8 ± 1.6
PPARGC1A 2962	GG	47.9 ± 12.2	51.5 ± 12.9	0.93 ± 0.19	0.99 ± 0.15	230.5 ± 7.1	217.7 ± 26.2	4.4 ± 1.6	4.7 ± 1.5
	GA	49.3 ± 8.7	51.7 ± 7.8	0.89 ± 0.17	1.03 ± 0.14	240.3 ± 37.9	224.9 ± 18.9	3.8 ± 1.1	4.1 ± 1.2
	AA	47.4 ± 11.7	50.1 ± 10.3	0.97 ± 0.24	1.01 ± 0.19	229.2 ± 54.3	220.4 ± 29.2	4.3 ± 1.0	4.9 ± 1.3
PPARA L162V	LL	48.4 ± 11.2	51.2 ± 10.4	0.94 ± 0.21	1.00 ± 0.16	233.1 ± 48.8	220.4 ± 25.6	4.0 ± 1.2	4.4 ± 1.3 **
	LV	47.9 ± 4.9	49.9 ± 5.2	0.84 ± 0.10	0.94 ± 0.09	240.6 ± 21.4	229.3 ± 11.4	4.4 ± 0.9	5.3 ± 1.4 **
PPARA Intron 7	CC	49.4 ± 8.3	52.4 ± 7.9	0.93 ± 0.20	1.00 ± 0.16	233.1 ± 43.3	222.5 ± 22.6	4.0 ± 1.2	4.5 ± 1.2
	CG	46.8 ± 12.7	49.2 ± 11.5	0.92 ± 0.21	0.99 ± 0.15	235.8 ± 51.2	220.6 ± 27.2	4.1 ± 1.2	4.7 ± 1.5
PPARD 294	TT	46.9 ± 12.1	49.5 ± 11.5	0.93 ± 0.24	0.98 ± 0.15	237.0 ± 50.8	220.2 ± 25.0	3.9 ± 1.2	4.3 ± 1.1
	TC	49.9 ± 6.6	52.9 ± 6.6	0.92 ± 0.16	1.00 ± 0.14	233.9 ± 41.1	233.9 ± 21.8	4.3 ± 1.1	4.8 ± 1.4
	CC	45.9 ± 15.1	47.9 ± 12.6	094 ± 0.26	1.01 ± 0.24	234.1 ± 77.0	216.5 ± 34.4	3.6 ± 1.2	4.2 ± 1.4
NRF2 A/C	CC	50.1 ± 6.1	52.4 ± 5.1	0.86 ± 0.12	1.01 ± 0.17	237.6 ± 33.4	222.6 ± 20.5	4.5 ± 0.7	4.7 ± 0.8
	CA	46.5 ± 11.8	49.6 ± 12.0	1.01 ± 0.27	1.03 ± 018	222.7 ± 54.5	216.6 ± 30.7	4.0 ± 1.0	4.6 ± 1.3 **
	AA	48.9 ± 10.7	51.6 ± 9.2	0.91 ± 0.17	0.97 ± 0.13	240.0 ± 42.9	224.4 ± 21.0	3.9 ± 1.3	4.5 ± 1.5 **

DT, deceleration time of early mitral flow; LVEF, left ventricular ejection fraction; MET, metabolic equivalents. * Significantly lower than Gly/Ser genotype. $P<0.05$; ** Significantly higher than baseline. $P<0.001$

Discussion

Identification of candidate genes that influence the response of cardiac patients to endurance training is of potential clinical applicability. This might help identify 'hypo' and 'hyperresponders' to cardiac rehabilitation programs, allowing for an optimization of training loads. Our main findings were: (1) *PPARGC1A* Ser482 allele homozygotes had lower baseline LVEF, (2) exercise training attenuated differences in LVEF between *PPARGC1A* Gly482Ser genotypes, (3) *PPARA* 162Val allele carriers showed a greater improvement in exercise tolerance after exercise training than 162Leu homozygous patients, and (4) *NRF2* A allele carriers improved exercise tolerance after exercise training, while it remained unchanged in patients with the CC genotype.

Several common gene variations have been discovered in the *PPARGC1A* gene. One of these variants results from the transition between amino acids glycine and serine in the residue 482 (Gly482Ser) (39). This gene variant has been associated with metabolic and cardiovascular disorders in humans such as diabetes (12) and hypertrophic cardiomyopathy (57). One common finding among these studies is that the 482Ser allele was the 'unfavourable' allele, i.e. associated with the disease (12, 57). This is consistent with our results. In the present investigation, patients with two copies of the 482Ser allele presented reduced left ventricular systolic function at baseline. The reasons that make these individuals more prone to heart failure or to a worse condition once heart failure develops are not clear, but several explanations can be proposed. The minor 482Ser allele is associated with reduced *PPARGC1A* mRNA levels (36). The deletion of *PPARGC1A* gene in mice results in abnormal substrate metabolism and reduced expression and function of proteins that are required for substrate utilization, mitochondrial function and oxidative phosphorylation (3, 35). These animals have also profound cardiac dysfunction (3, 35), increased transcription of inflammation mediators, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, as well as increased production of reactive oxygen species (ROS) that parallels the reduced expression of anti-oxidant enzymes (24). Thus, one might presume that 482Ser allele homozygotes are more predisposed to lower cardiac metabolism reserve, abnormal mitochondrial function, inflammation and significant cardiac dysfunction due to reduced gene expression.

The current study also showed that training-induced improvement in LVEF is associated with the *PPARGC1A* Gly482Ser variant. Endurance training has been shown to increase the cardiac expression of *PPARGC1A* (17, 33) as well as cardiac mitochondrial respiration and fatty acid oxidation in experimental heart failure (23, 44). In addition, these changes occurred in concert with increased cardiac contractility (23, 33). Thus, a possible explanation for our findings could be an increase in the cardiac expression of *PPARGC1A*. Direct evidence to confirm that this occurs in the human heart is unlikely to become available. Hence, further studies should replicate the association between the *PPARGC1A* Gly482Ser variant and training-induced improvements in left ventricular function observed in the present study.

This investigation failed to observe any association between diastolic function and gene variants either at baseline or in response to exercise training. This is in apparent contrast with a recent report, in which the *PPARGC1A* Gly482Ser variant was associated with diastolic dysfunction in men, but not in women (28). However, this association was determined by examining the allele distribution in men with and without diastolic dysfunction (28), while here we measured diastolic dysfunction in heart failure patients.

The present investigation indicates that *PPARA* gene variants could also be candidates to influence training responses in heart failure patients. PPARα (which is encoded by *PPARA*) is a ligand-activated transcription factor that regulates the expression of genes involved in fatty acid uptake and oxidation and lipid metabolism (30). Thus, it plays a prominent role in controlling energy supply to the heart and skeletal muscles. Patients with terminal heart failure have been found to have lower PPARα levels compared to donor hearts (31), indicating that energy provision is altered in heart failure. A common substitution of a leucine for a valine (Leu162Val) in the human *PPARA* gene (56) has been shown to alter the function of this transcription factor (19, 48). Our data show that this variation is associated with exercise tolerance in response to exercise training in heart failure patients. We found that exercise tolerance improved more pronouncedly in 162Val carriers than in 162Leu homozygous patients. Functional studies have demonstrated that transactivation of the receptor in vitro is greater in the presence of the 162Val allele (19), and that this activation is dependent on ligand concentrations (48). Thus, it can be presumed that

the 162Val allele increases receptor activity under conditions of enhanced lipid concentrations, such as during and following aerobic exercise (45, 46). If this notion applies to chronic exercise, which has been shown to increase muscle expression of PPARα along with proteins involved in fatty acid oxidation and glucose transport (52, 53), patients with the 162Val allele may benefit from enhanced fatty acid oxidation and less decline of glycogen stores during exercise. This could delay the onset of fatigue and explain the more pronounced improvement in exercise tolerance.

Endurance exercise also increases muscle respiratory capacity (18), which is associated with increases in mitochondrial density and content (18). This is attributed in part to exercise-induced increases in muscle NRF1 and NFR2 (6). These transcription factors control the transcription of genes encoding mitochondrial proteins, including cytochrome c, components of all five electron transport chain complexes, mitochondrial import proteins, and proteins required for heme biosynthesis (32). In addition, NRF1 and NRF2 regulate mitochondrial transcription factor A (TFAM), a nuclear-encoded transcription factor essential for replication and transcription of mitochondrial DNA (20). These data indicate that NRF2 is critical for the occurrence of mitochondrial adaptations to exercise training.

This study found that a particular *NRF2* SNP (rs12594956) is associated with the training response in heart failure patients. The association between training response and *NRF2* gene variants has been suggested in a previous genome wide scan investigation (8). Moreover, various NRF2 gene variants, including this one, have been associated with elite endurance performance, which relies mainly on aerobic metabolism (13, 15). In addition, our results are consistent with those of a study by He *et al* (27) reporting that men who carried the *NRF2* ATG haplotype had 57.5% higher running economy in response to exercise training compared with other haplotypes. Thus, these data suggest that the *NRF2* A/C variant, alone or in combination with other *NRF2* variants influences the magnitude of the training response in heart failure patients. However, it is unknown whether this variant is functional or whether it is in linkage disequilibrium with other functional variants. In addition, this variant is located in an intron region and it is not known whether noncoding mutations in *NRF2* gene might influence the alternative splicing of transcripts, and modulate NRF2 expression and mitochondrial biogenesis (27).

Therefore, functional studies are needed to elucidate the molecular mechanisms that *NRF2* gene variants orchestrate to modulate endurance performance.

We believe the results of our study are overall valid, as all the following criteria were met (5): phenotypes were accurately assessed, subjects were ethnically-matched, genetic assessment was accurate, reliable and unbiased, genotype distributions were in Hardy-Weinberg equilibrium, and our results are in line with previous research in the field (27, 57). However, the low sample size of our cohort does considerably limit the 'external validity' (and therefore generalizability) of our results. We believe this limitation could be partly overcome by the fact that the study phenotypes were consistently and reliably assessed by the same researchers. This eliminates a potential confounder that exists in most multi-centre studies with larger samples, i.e. phenotype data are assessed by different investigators and analyzed together, yielding a considerable source of variability. The lack of data from a 'replication' cohort of a different ethnic background is also to be kept in mind. Thus, more research is needed using our model on larger groups of different ethnic backgrounds. On the other hand, we believe there are strengths in our design. It is indeed difficult to recruit large samples of heart failure patients for long-term exercise training programs (9, 41). In the present study, we included heart failure patients with reduced and preserved LVEF. Similar to other reports (11, 34, 58), we showed that exercise training could improve diastolic dysfunction and exercise tolerance in both subsets of heart failure (1). Moreover, abnormal cardiac metabolism has been shown in both subsets of heart failure (29, 43). In addition, our results did not change after adjusting for heart failure subsets.

In conclusion, the current study showed that the *PPARGC1A* Gly482Ser variant is associated with systolic dysfunction at baseline and its improvement in response to exercise training in heart failure patients. In addition, *PPARA* Leu162Val and *NRF2* A/C gene variants are associated with the training response of exercise tolerance in heart failure patients.

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CHAPTER IV

OVERALL DISCUSSION

Methodological discussion

In contrast to a number of previous studies (Gary, et al., 2004; Small, Wagoner, Levin, Kardia, & Liggett, 2002), the present studies randomized heart failure patients to a supervised exercise training program or usual care alone (studies I and II). This design is the most appropriate to ascertain the effects of exercise training relative to those promoted by standard medical care on the evolution of cardiac function and exercise tolerance over time. In addition, these studies involved not only heart failure patients with moderate to severe systolic dysfunction at rest, but also those with mild or even preserved left ventricular ejection fraction (LVEF). At the time this thesis was planned, no randomized controlled trials had been conducted to examine the effects of exercise training on the ventricular function in heart failure patients with preserved LVEF (HFPEF). This issue is important for several reasons. These patients comprise almost half of the heart failure population (Hogg, Swedberg, & McMurray, 2004). Thus, studies hitherto have ignored the potential impact of exercise training in almost half of the heart failure population. Furthermore, these patients are more often female, older and obese (Hogg, et al., 2004; Maeder & Kaye, 2009). These characteristics are important predictors of low participation in exercise training programs (Corra, Mezzani, Giordano, Caruso, & Giannuzzi, 2011; Worcester, Murphy, Mee, Roberts, & Goble, 2004). Thus, the current approach allows us to evaluate the effectiveness of exercise training as a valid treatment in the whole range of heart failure patients.

The European Society of Cardiology (ESC) has proposed three main criteria for the diagnosis of heart failure, i.e., signs or symptoms typical of heart failure and objective evidence of cardiac functional or structural abnormalities at rest (Dickstein, et al., 2008). These criteria were adopted for the inclusion of patients in all our studies. Furthermore, a consensus statement from experts representing the same organization (ESC) extended these criteria for the diagnosis of HFPEF (Paulus, et al., 2007). Thus, in line with these recommendations, our patients had to present (i) normal or mildly abnormal systolic LV function and (ii) evidence of diastolic LV dysfunction (Paulus, et al., 2007).

Cardiac magnetic resonance is considered to be the gold standard for the assessment of left ventricular systolic function, volumes, mass and wall thickness due to its high spatial resolution, signal-to-noise ratio and reproducibility (Leong, De Pasquale, & Selvanayagam, 2010). However, its elevated cost limits serial assessments of left ventricular function and structure and its application in clinical practice. Thus, in our studies (I-IV), left ventricular structure and function were assessed through echocardiography. In particular, left ventricular ejection fraction (LVEF, %) was measured to assess the effects of exercise training (studies I-II), single nucleotide polymorphisms and the interaction between both (studies III-IV) on left ventricular systolic function. This parameter is associated with prognosis in heart failure (Jorge Alves, et al., 2010) and most clinical trials and exercise training studies to date have used it to assess the effectiveness of treatment on systolic dysfunction (Abraham, et al., 2002; Cohn & Tognoni, 2001; Ghio, et al., 2006; Haykowsky, et al., 2007; St John Sutton, et al., 2003). However, its large inter- and intra-observer variability recommends caution in the interpretation of results obtained with this method (Galderisi, et al., 2011; Himelman, Cassidy, Landzberg, & Schiller, 1988). The lack of intra-observer reproducibility measures could be considered a limitation of our studies. Nonetheless, all echocardiographic evaluations were performed by the same physician, and given his blindness to the treatment assignment, we are confident that evaluation bias did not influence the outcomes of this investigation.

Diastolic function was assessed through transmitral inflow velocities [early mitral flow velocity (E), late or atrial mitral flow velocity (A), and the ratio between them (E/A)] and E-wave deceleration time (studies I-IV). The importance of these parameters has been confirmed in several clinical trials, which have shown for example that deceleration time is a strong predictor of prognosis in patients with heart failure (Giannuzzi, et al., 1996; Xie, Berk, Smith, Gurley, & DeMaria, 1994). This parameter has also been associated with collagen volume fraction and cross-linking measured in myocardial biopsies taken from patients with HFPEF (Kasner, et al., 2011).

Tissue Doppler imaging (TDI)-derived mitral annular velocities have been recommended for the evaluation of diastolic function on the basis of their lower sensitivity to loading conditions (Paulus, et al., 2007). The ratio of early mitral

transmitral flow velocity with early diastolic velocity of the mitral valve annulus (E/E') has been associated with left ventricular filling pressures and has been shown to be a strong predictor of survival (Hillis, et al., 2004; Ommen, et al., 2000). The value of E/E' as a predictor of intracardiac filling pressures in patients with decompensated advanced heart failure was questioned in a recent investigation (Mullens, Borowski, Curtin, Thomas, & Tang, 2009). Nonetheless, this parameter would certainly be of value in our studies for the assessment of diastolic function and estimation of left ventricular filling pressures.

LV diameters derived from M-mode allow accurate estimation of chamber size in ventricles with symmetric contraction. However, cardiac diameters provide only a rough indication of cardiac remodelling in heart failure patients. The evaluation of LV volumes would be preferable; more so in patients with distorted left ventricles and wall motion abnormalities (Galderisi, et al., 2011).

The objective assessment of exercise tolerance requires the determination of peak oxygen consumption. However, due to technical limitations, treadmill speed and grade were recorded and used to estimate metabolic equivalents (METs) as indicators of exercise tolerance. This method has been used in previous studies with heart failure patients as it shows a good correlation with peak oxygen consumption.

Exercise training consisted of aerobic interval training of moderate intensity. Numerous studies have designed similar programmes with some nuances and concluded that moderately intense aerobic exercise training can improve exercise tolerance and cardiac function in heart failure patients (Giannuzzi, Temporelli, Corra, & Tavazzi, 2003; Hambrecht, Gielen, et al., 2000; O'Connor & Whellan, 2009). In a recent study, high-intensity interval training (90-95% peak heart rate) has been shown to be more effective in improving exercise tolerance, left ventricular function and cardiac remodelling than moderate continuous exercise training (70-75% peak heart rate) (Wisloff, et al., 2007). There were no reported exercise-related events. Nonetheless, large trials are needed to confirm that high-intensity interval training programmes are safe in heart failure patients.

In contrast to previous studies (Edelmann, et al., 2011; Kitzman, Brubaker, Morgan, Stewart, & Little, 2010; Smart, Haluska, Jeffriess, & Marwick, 2007), we did not evaluate health-related quality of life. This is a very important issue for elderly

patients (Kitzman, 2011), such as those included in our studies. Thus, assessing quality of life would be important to understand the impact of exercise training on our patients' lives (studies I and II).

Even though these considerations apply to all studies that compose this dissertation, some limitations can be ascribed to studies III and IV. The most important limitation of these two studies is their small sample size. A group of experts concluded that almost 800 subjects are needed to test the association between a single nucleotide polymorphism and a complex human trait, assuming that it accounts for 1% of the trait variance (with minimal acceptable power) (Hagberg, et al., 2011). This number increases if the expected trait variance decreases and the *P* value threshold for significance decreases to account for multiple testing (Hagberg, et al., 2011). Moreover, this panel of investigators advocate that sample size must increase several fold when the contribution of an interaction term to the trait variance is under investigation. On the other hand, our study design (III and IV) is different from the classic case-control studies on which these calculations were based. Our own power analysis using G-power analysis software (Faul, Erdfelder, Lang, & Buchner, 2007) indicates that almost 1000 patients would be required to detect a small effect size ($f=0.10$) between three genotype groups with minimal acceptable power (0.80). In contrast, our analysis reveals that 250 patients would be enough to detect a small effect ($f=0.10$) in a repeated measures genotype x time interaction design. This number increases to almost 400 patients if we decrease the *P* value threshold for significance to account for multiple testing ($\alpha=0.008$). Thus, these data indicate that studies III and IV were underpowered. Thus, since small studies are prone to large variation in risk estimates or trait variance, further studies with different and larger cohorts are encouraged to confirm our results (Chanock, et al., 2007).

In addition, the analysis in studies III and IV included patients with HFREF and HFPEF. Considering the differences between these two subsets, the pooled analysis may have introduced bias in the association between single nucleotide polymorphisms, alone or in interaction with exercise training, and left ventricular function (LVEF). On the other hand, since abnormalities in left ventricular diastolic function and exercise tolerance are more uniform between these two subsets, we are

more confident that the pooled analysis had little influence on these findings (studies III-IV).

To understand the biological role of a single nucleotide polymorphism, it is useful to conduct functional genotype studies. These studies serve to clarify whether and how certain variants affect the expression or function of their corresponding genes. Therefore, conscious of our inability to conduct such studies, we chose as much as possible single nucleotide polymorphisms that have been demonstrated to be functional or associated with a trait of interest (biological plausibility).

Discussion of the main results

The main findings of the studies that composed this dissertation indicate that exercise training, single nucleotide polymorphisms and the interaction between both play important roles in the modulation of left ventricular function and exercise tolerance in heart failure patients with or without reduced left ventricular ejection fraction (LVEF). Our data from studies I and II showed that exercise training improves left ventricular function and exercise tolerance not only in heart failure patients with reduced ejection fraction (HFREF) but also in those with preserved ejection fraction (HFPEF). Furthermore, studies III and IV demonstrated that single nucleotide polymorphisms involved in beta-adrenergic signalling transduction and muscle metabolism are associated with left ventricular function and exercise tolerance measured before and after exercise training. These results suggest that an intricate process involving multiple interactions between individual genetic characteristics and environmental cues determines the clinical status of heart failure patients.

Effect of exercise training on left ventricular function and exercise tolerance in heart failure patients

It is well known that endurance training can improve ventricular function, attenuate cardiac remodelling and increase exercise tolerance in heart failure patients (Davies, et al., 2010; Haykowsky, et al., 2007; O'Connor & Whellan, 2009). These changes with training are important because these parameters are strong predictors of prognosis (Giannuzzi, et al., 1996; Jorge Alves, et al., 2010). Our data (study I) showed that exercise training also improves cardiac function and exercise tolerance in patients with HFPEF. These observations are in line with those reported in a recent investigation (Edelmann, et al., 2011). Edelmann et al. (2011) reported that endurance combined with resistance training improves diastolic function, exercise tolerance and quality of life in patients with similar characteristics as those that were included in our studies (I and II). The results of both studies indicate that moderate intensity endurance training alone or in combination with resistance training is effective in improving exercise tolerance and diastolic function in patients with HFPEF.

Furthermore, a recent randomized controlled trial found improvements in exercise tolerance (Kitzman, et al., 2010). Patients included in this trial had similar characteristics (LVEF ≥50%), and the intensity of endurance training (60-70% of HR reserve) and the magnitude of improvement in the exercise tolerance (16%) were also similar between this and our studies (I and II). However, no changes in cardiac function were found following exercise training. The reasons for these contrasting results are not clear, but a number of potential explanations are proposed. Patients included in the study by Kitzman et al. (2010) were older (70 ± 6 yrs vs. 62 ± 10 yrs) and exercised for a shorter period of time compared with our patients (16 weeks vs. 24 weeks). Thus, longer exercise training programmes could be necessary to improve cardiac function in older individuals. On the other hand, in the same study (Kitzman, et al., 2010) both exercise and usual care groups changed diastolic function and decreased ejection fraction over time. Thus, experimental procedures or pharmacological intervention could account for these observations. Taken together, these data indicate that moderate exercise training improves exercise tolerance in patients with HFPEF. However, the impact of exercise training on cardiac function remains to be elucidated.

To confirm this notion, we repeated this investigation in patients with preserved, mild and moderate to severe systolic dysfunction (study II). Our data showed that exercise training improves left ventricular function and exercise tolerance in all heart failure subsets. An overwhelming body of evidence shows that endurance training increases exercise tolerance in heart failure patients (Belardinelli, Georgiou, Cianci, & Purcaro, 1996; Davies, et al., 2010; Erbs, et al., 2010; Hambrecht, Hilbrich, et al., 2000; O'Connor & Whellan, 2009; van Tol, Huijsmans, Kroon, Schothorst, & Kwakkel, 2006). Furthermore, endurance training has been shown to improve resting and peak systolic function in patients with severe systolic dysfunction (Belardinelli, et al., 1996; Coats, et al., 1992; Delagardelle, et al., 2008; Erbs, et al., 2010; Hambrecht, Gielen, et al., 2000; Hambrecht, et al., 1995; Klecha, et al., 2007). Even though these observations derived from small studies, a recent meta-analysis confirmed that endurance training has a favourable impact on systolic dysfunction in patients with HFREF (Haykowsky, et al., 2007). Evidence has emerged that systolic dysfunction improves even following combined endurance-resistance training (Beckers, et al.,

2008). A smaller number of randomized controlled studies reported that endurance training also improves diastolic function in heart failure patients (Belardinelli, et al., 1996; Myers, et al., 2002; Wisloff, et al., 2007). Moreover, in line with our results (study II), exercise training has been confirmed to attenuate cardiac remodelling in heart failure patients with significant left ventricular dilatation (Giannuzzi, et al., 2003; Hambrecht, Gielen, et al., 2000; Haykowsky, et al., 2007). However, our results were modest in patients with mild as well as moderate to severe systolic dysfunction, perhaps because left ventricular dilatation was less extensive than that reported in other patients (Giannuzzi, et al., 2003; Hambrecht, Gielen, et al., 2000). Furthermore, cardiac diameters did not improve in patients with preserved ejection fraction, which may stem from concentric remodelling. Nonetheless, without measuring left ventricular mass, one cannot be certain. Thus, together with our findings, these data strongly indicate that exercise training improves left ventricular dysfunction in heart failure patients. It also suggests that exercise training may attenuate cardiac remodelling. However, it should be noted that a number of studies have failed to replicate these findings (McKelvie, et al., 2002; Myers, et al., 2002; Parnell, Holst, & Kaye, 2002). The reasons for these contrasting results are not clear, but a number of possible explanations are advanced in this discussion.

The absence of clear guidelines for the implementation of exercise training programmes in heart failure patients has led to wide variations in setting (home based versus supervised), duration, frequency, exercise modality and intensity, fitness equipment and exercise testing protocols (Conraads & Beckers, 2010; Meyer, Kindermann, & Kindermann, 2004; Piepoli, et al., 2011). These variations make the comparison between studies very difficult. In most studies, training programmes consisted of continuous endurance exercise, 3 (to 5) times a week for 3 months (Conraads & Beckers, 2010; Meyer, et al., 2004; Piepoli, et al., 2011). Studies have used varied exercise intensities, although 70-80% of peak VO_2 or HR has been prescribed in most clinical studies (Conraads & Beckers, 2010; Meyer, et al., 2004; Piepoli, et al., 2011). It remains unclear how training characteristics influence the success of exercise training programmes, but evidence from individual studies and meta-analysis has provided some insights. For example, low-intensity exercise training (40 to 60% of peak VO_2) has been shown to improve exercise tolerance but not

cardiac output or stroke volume in heart failure patients (Belardinelli, Georgiou, Scocco, Barstow, & Purcaro, 1995). On the other hand, several studies have shown that moderate intensity endurance training (70-80% of peak VO_2) not only increases exercise tolerance but also improves resting, submaximal and peak left ventricular systolic function (Coats, et al., 1992; Dubach, et al., 1997; Giannuzzi, et al., 2003; Hambrecht, Gielen, et al., 2000; Hambrecht, et al., 1995; Klecha, et al., 2007). Thus, moderate intensity exercise training seems to be necessary to induce positive modifications in left ventricular dysfunction. Nonetheless, high-intensity interval training (95% of peak HR) has been shown to be more effective than moderate continuous exercise (70% of peak HR) for improving cardiovascular and skeletal muscle function (Wisloff, et al., 2007). Moreover, exercise earlier after myocardial infarction (around 1 week) and for longer periods of time (6 months) has been suggested to result in greater benefits in cardiac function and remodelling (Belardinelli, et al., 1995). In this line of reasoning, the volume of exercise training (the product of exercise duration, number of sessions and duration of the training period) has been contended to influence the benefits of training (Crimi, Ignarro, Cacciatore, & Napoli, 2009). For example, smaller increases in exercise tolerance have been documented in exercise training programmes of short duration (less than 2 months) compared with longer exercise training programmes (3 to 6 months) (Harris, LeMaitre, Mackenzie, Fox, & Denvir, 2003; Jette, Heller, Landry, & Blumchen, 1991; Meyer, et al., 2004). However, there seems to be a time or training dose after which improvements with exercise training may decrease (Meyer, et al., 2004). Increases in exercise capacity are more pronounced during the first (2 to 3) months of exercise training (Meyer, et al., 2004), but further improvements in exercise tolerance, cardiac function and remodelling have been observed in long-term (6-month) exercise training programmes (Giannuzzi, et al., 2003; Hambrecht, Gielen, et al., 2000; Hambrecht, et al., 1995). Hence, long-term exercise training seems to be required to achieve the greatest benefits in cardiovascular and skeletal muscle function. Furthermore, the frequency of exercise may also play an important role in the impact of an exercise-training programme, since studies that instructed their patients to exercise daily reported above-average improvements in exercise tolerance (Dubach, et al., 1997; Hambrecht, et al., 1995). Together, these data indicate that long-term moderate to high intensity exercise training produces the greatest benefits in exercise

capacity, left ventricular function and left ventricular remodelling in heart failure patients.

The multicentre randomized controlled HF-ACTION (Heart Failure: A Controlled Trial Investigation Outcomes of Exercise Training) trial provided valuable information to elucidate the influence of exercise training characteristics in heart failure patients. This trial included patients with severe systolic dysfunction (LVEF≤35%), among which 1159 patients were allocated to the training group. Following three months of exercise training, mean peak VO_2 increased 0.6 $ml.kg^{-1}min^{-1}$, a value much lower than that reported in previous studies (~ 2.1 $ml.kg^{-1}min^{-1}$) (Meyer, et al., 2004; van Tol, et al., 2006). This discrete outcome has pointed to the main weakness of long-term exercise programmes: compliance to long-term treatment. Indeed, only 40% of the patients met the recommended training target of 90 minutes per week during the first three months, and 120 minutes per week during the following months (O'Connor & Whellan, 2009). The importance of compliance to the outcomes of exercise training programmes was confirmed in this trial, as the volume of exercise completed each week was associated with improvements in exercise capacity, quality of life, hospital admissions and survival. The authors showed that patients who completed 6 METs-hour per week (i.e., walking 25 minutes at 2.5 mph, 5 times a week) increased exercise capacity and decreased risk of cardiac events compared with those who did not. These data support our observations (studies I-II), which resulted from a training programme with a reasonable volume of exercise.

Besides the training features, psychological disturbances, physical comorbidities and socioeconomic factors may influence compliance with exercise training. Thus, our findings (studies I-II) may not be generalizable to all patients, as conditions that limit participation in exercise were considered to be an exclusion criterion. On the other hand, training characteristics, compliance or physical comorbidities cannot explain the widely variable individual responses to exercise training that were observed in our studies (I-II). Thus, genetic factors may play an important role in the response to cardiac rehabilitation (studies III-IV).

Effect of genotype on left ventricular function and exercise tolerance in heart failure patients

In the last decades, the discovery of genotypes that might predispose individuals to develop heart failure has been pursued by many investigators (Small, et al., 2002; Smith, et al., 2010). A number of genetic variants have been discovered to be more prevalent in heart failure patients than in controls (Forleo, et al., 2007; Raynolds, et al., 1993; Smith, et al., 2010), leading researchers to conclude that common mutations were associated with an increased predisposition to heart failure. However, replication of these positive associations has often failed in independent studies (Alves, Eynon, Oliveira, & Goldhammer, 2010). Failure to detect an association has been attributed in part to the reductionist assessment of isolated single nucleotide polymorphisms (SNPs) (McNamara & London, 2010). Whether genetic factors contribute to the predisposition to and consequences of cardiovascular diseases is beyond question (Zdravkovic, et al., 2002). Recent estimates point to a significant contribution (~18%) of genetic factors to the development of heart failure (Lee, et al., 2006). The difficulty is that gene variants are likely to predispose to complex diseases such as heart failure not alone but in combination with other gene variants and with the environment (Goldstein & Cavalleri, 2005). Thus, studies that were able to assess thousands if not millions of SNPs at the same time, such as those using genome-wide association scans (GWAS), were widely welcomed (McNamara & London, 2010). However, the need for large sample sizes and conservative correction for multiple genetic markers have often led to modest results (Morrison, et al., 2010). This was illustrated in a recent large-scale investigation (Smith, et al., 2010). This investigation included more than 23.000 individuals, among whom almost 3000 developed heart failure during a 13-year follow-up, and assessed almost 2.5 million markers. Among these, only two genetic markers exceeded the genome-wide threshold for significance (5.0×10^{-7}). One SNP was found in individuals with European ancestry and was located near the ubiquitin-specific protease 3 gene (USP3). The other one was detected in individuals of African ancestry and was located close to the leucine-rich repeats and immunoglobulin-like domains 3 gene (LRIG3).

The results of this investigation are in contrast with the candidate gene approach, which devoted its efforts to investigating genes that are presumably involved in the

pathogenesis of heart failure, such as those of the adrenergic receptors, renin-angiotensin-aldosterone system (Raynolds, et al., 1993; Small, et al., 2002), calcium regulatory proteins (Haghighi, et al., 2008; Schmidt, et al., 2003) inflammatory proteins (Tiret, et al., 2000), endothelial function regulators (Tiret, et al., 2000) matrix metalloproteinases (Mizon-Gerard, et al., 2004) and proteins of the cytoskeleton (Liew & Dzau, 2004). Thus, much more research is needed to elucidate which and how genetic variants predispose to common diseases such as heart failure.

A stronger case has been made for the clinical relevance of identifying the genetic predictors of a patient's response to treatment (Goldstein & Cavalleri, 2005). Identification of 'unfavourable' alleles may be of clinical relevance because it may facilitate the identification of patients who may benefit from earlier aggressive treatment. Genetic variations seem to function as 'modifiers', i.e., modulating the consequences of heart failure (Alves, et al., 2010; Biolo, et al., 2008; McNamara, et al., 2004). For example, various SNPs located in the adrenergic receptor genes have been associated with reduced basal and agonist-stimulated inotropic and lusitropic responses in heart failure patients (Barbato, et al., 2007; Scharin Tang, Lindberg, Gruner Svealv, Magnusson, & Andersson, 2007; Wolk, et al., 2007). Thus, patients with putatively 'unfavourable' alleles might benefit from earlier aggressive treatment (Liggett, et al., 1998). Indeed, some clinical trials have shown that patients with 'unfavourable' alleles, i.e., those associated with poorer cardiac function and survival, have the greatest benefits with pharmacological treatment (Barbato, et al., 2007; Chen, et al., 2007; Liggett, et al., 2008; Liggett, et al., 2006; Liggett, et al., 1998; McNamara, et al., 2001). Furthermore, Cresci et al. (2009) reported that treatment was more effective in heart failure patients who carried unfavourable alleles in two different genes: ADRB1 and GRK5. These data suggest that outcomes in heart failure are modulated by complex gene-gene and gene-environment interactions.

Thus, identification of genetic markers of cardiac dysfunction constitutes an important step to understanding the molecular mechanisms that govern the individual response to treatment in heart failure patients (Barbato, et al., 2007; Tang, et al., 2002; Wolk, et al., 2007). One of these potential 'unfavourable alleles is the GNAS -1211G allele that was associated with worse diastolic function. The -1211G-A allele transition has been shown to alter transcription factor binding and promoter activity (Frey, et al., 2009;

Frey, et al., 2008). Thus, one might presume that the -1211G allele may contribute to depression in intracellular adrenergic signalling, calcium regulation and diastolic function in heart failure patients (Ikeda, Y., Hoshijima, & Chien, 2008). The other potential variant is the *PPARGC1A* Ser482Ser, which was associated with significant systolic dysfunction in heart failure patients. Studies in humans and knockout animals support the notion that *PPARGC1A* plays an important role in heart failure (Ingwall, 2009; Ventura-Clapier, Garnier, Veksler, & Joubert, 2011). There is evidence to support that *PPARGC1A* controls cardiac metabolism, mitochondrial biogenesis and inflammation (Arany, et al., 2005; Lehman, et al., 2008). This is consistent with the cardiac metabolic, mitochondrial, inflammatory, and functional abnormalities that are observed in mice without the *PPARCGC1A* gene (Lehman, et al., 2008; Leick, et al., 2008). The mechanisms that govern this gene association are not clear but may stem from the reduced expression of *PPARGC1A* (Ling, et al., 2004).

These data suggest that the SNPs studied here are putatively unfavourable to heart failure patients, but these results are hampered by the small sample size and small number of studied variants in both studies (I-II). Thus, more research in warranted in larger and different cohorts to confirm our findings.

Similar to previous pharmacogenetic studies (Chen, et al., 2007; Terra, et al., 2005), exercise training attenuated cardiac dysfunction in patients with presumably 'unfavourable' alleles. More importantly, the 'discriminatory' effect of exercise training attenuated the genotype differences that were noticed at baseline. These data give credence to the notion that patients with worse clinical status are likely to benefit the most from exercise training.

Effect of genotype-exercise training interaction on left ventricular function and exercise tolerance in heart failure patients

Even though several studies have demonstrated that exercise training offsets the maladaptive process of heart failure (Haykowsky, et al., 2007), considerable inter-individual variation is noticed (Hambrecht, Gielen, et al., 2000). These observations are in agreement with the current findings, i.e., exercise training increased mean exercise tolerance, but not all patients showed clinically significant improvements (studies I and II). These findings indicate that the response to exercise training varies

among individuals, so that exercise does not seem to be uniformly effective, and imply that genetic factors may affect the therapeutic response to exercise (studies III and IV).

Identification of 'favourable' genotypes in response to endurance training, i.e., those allowing for attainment of the greatest possible gains, might be of potential clinical relevance for cardiac patients. In other words, identification of low and high responders to cardiac rehabilitation programs might allow for an optimization of training loads and/or search for alternative therapies. However, substantial variation is known to exist between individual genomes (Goldstein & Cavalleri, 2005). This makes identification of genotypes that are associated with multifactorial human traits such as heart failure a daunting task. It is the combination of variants within a gene (haplotype) and between genes and the interaction of these combinations with the environment that determines a given phenotype. Indeed, reported estimates indicate that almost 50% of variation in maximal oxygen consumption (VO_2max) in untrained state and in response to exercise training is attributable to genetic variation (heritability) (Bouchard, et al., 1999; Bouchard, et al., 1998). Human training response in turn is determined by an intricate cooperation between several intermediate phenotypes, for example cardiac function, pulmonary reserve, skeletal muscle strength and vasomotor response (Puthucheary, et al., 2011). These intermediate phenotypes in turn are the consequence of a complex interaction between anatomical, functional, neuronal, and biochemical factors, each of which is under the influence of genetic variants and their interaction with the environment (Montgomery & Safari, 2007). One good example is the heart, whose function depends on chamber dimensions, wall thickness, coronary blood flow, vasomotor response, adrenergic receptors, calcium handling, fatty acid and glucose metabolism, mitochondrial density and content and oxidative phosphorylation, to name but a few (El-Armouche & Eschenhagen, 2009; Mann & Bristow, 2005; Montgomery & Safari, 2007; Neubauer, 2007). Given that individual genes influence each of these phenotypes, it is not surprising that more than 200 variants have been associated with human performance (Bray, et al., 2009). In our studies (III and IV), three genetic variants were associated with exercise tolerance in response to exercise training in heart failure patients, including one related to the control of fatty acid metabolism

(*PPARA* Leu162Val), one coding for adrenergic receptors (*ADRB1* Arg389gly) and another one related to mitochondrial biogenesis (*NRF2* A/C). There is evidence based on in vitro and in vivo experiments and/or case-control studies to support the association of these variants with the exercise tolerance response to chronic exercise (Baar, et al., 2002; Handschin & Spiegelman, 2008; He, et al., 2007; Liggett, et al., 2006; Mason, Moore, Green, & Liggett, 1999; Mialet Perez, et al., 2003; Sapone, et al., 2000; Terada, Kawanaka, Goto, Shimokawa, & Tabata, 2005). Hence, these variants may confer some advantage in patients that engage in cardiac rehabilitation programs.

The results of a recent genome wide scan association study pointed to another direction (Bouchard, et al., 2011). This investigation identified a panel of 21 SNPs accounting for 49% of the variance in VO_2max training response (Bouchard, et al., 2011). None of the variants identified in this study have been previously associated with the VO_2max training response (Bouchard, et al., 2011; Bray, et al., 2009). Moreover, their main findings were different from those identified in the same population using another method to predict the genetic component of VO_2max training response, i.e., analysis of mRNA expression signature in the skeletal muscle (Timmons, et al., 2010). The question is not whether genetic factors contribute to the variance in the human response to exercise training, but which genetic variants are involved in this process.

It is important to note that predisposition to exercise may also be genetically determined (Maia, Thomis, & Beunen, 2002; Moore, et al., 1991). Furthermore, there are a number of intrinsic and extrinsic factors influencing the individual's response to exercise training, e.g., technique, motivation, pain tolerance, depression, self-esteem, social support and lifestyle behaviours that may or may not be associated with genetic variation (Eynon, et al., 2011). On the other hand, the genetic component of the response to exercise training may not be reducible to nucleotide variability in the human genome. Evidence has emerged suggesting that micro RNAs (miRNAs) are important post-transcriptional regulators of gene expression by inducing translational repression or promoting mRNA degradation (gene silencing) (Eulalio, Huntzinger, & Izaurralde, 2008; Valencia-Sanchez, Liu, Hannon, & Parker, 2006). It has been estimated that each miRNA regulates hundreds of different mRNAs (Bushati & Cohen, 2007; Eulalio, et al., 2008). Indeed, various miRNA species have been shown

to be up- and down-regulated in animal models and human heart failure in tandem with altered expression of several hundred genes (Ikeda, S., et al., 2007; Thum, et al., 2007; van Rooij, et al., 2006). Furthermore, miRNAs have been shown to regulate key components of the remodelling process, including cardiomyocyte growth and apoptosis, extracellular matrix remodelling, and neurohormonal activation, suggesting that these small molecules have an active role in the pathogenesis of heart failure (Divakaran & Mann, 2008).

The potential role of miRNAs in the response to exercise training has been documented in recent studies (Davidsen, et al., 2011; Keller, et al., 2011). Exercise training has been shown to modulate the expression of various miRNA species in sedentary individuals (Keller, et al., 2011). Furthermore, increases in skeletal muscle mass following resistance exercise have been associated with selected changes in mRNAs expression (Davidsen, et al., 2011). Indeed, differential changes in the skeletal muscle expression of numerous miRNAs have been seen in low and high responders to resistance exercise (Davidsen, et al., 2011). Thus, these data indicate that miRNAs may play an important role in the inter-individual variability in the response to exercise training at least in healthy individuals. However, their potential role in heart failure patients is yet to be determined.

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CHAPTER V

CONCLUSIONS

Conclusions

Based on the general conclusions of each of the different studies that were presented in this thesis, it is possible to outline the following major conclusions:

Exercise training improves left ventricular function and exercise tolerance in heart failure patients with preserved as well as mild and moderate to severe reduction of left ventricular ejection fraction.

GNAS -1211G allele carriers may have elevated deceleration time of early mitral flow at baseline compared with -1211A allele homozygotes. On the other hand, exercise training seems to decrease the deceleration time in *GNAS* -1211G allele carriers, but not in -1211A allele homozygotes.

PPARGC1A Ser482 allele homozygotes may have reduced left ventricular ejection fraction at baseline compared with heterozygotes and Gly482 allele homozygotes. On the other hand, exercise training seems to increase left ventricular ejection fraction more pronouncedly in *PPARGC1A* Ser482 allele homozygotes and to attenuate their differences at baseline with respect to heterozygotes.

ADRB1 389Gly homozygotes seem to have a greater training-induced increase in exercise tolerance than 389Arg homozygotes. Nonetheless, metoprolol and atenolol appear to abolish the differences between *ADRB1* genotypes.

PPARA 162Val allele carriers show a greater training-induced improvement in exercise tolerance than 162Leu homozygous patients

NRF2 A allele carriers show improvements in exercise tolerance following exercise training, while C allele homozygotes appear to have no benefit from exercise training.

In summary, data from the present thesis indicate that exercise training improves left ventricular function and exercise tolerance in all heart failure subsets. Moreover, gene variants alone and in combination with exercise training appear to modulate left ventricular function and exercise tolerance in heart failure patients.