Benefit of Exercise Training ThErapy and cardiac Resynchronization in Heart Failure patients
(BETTER-HF)

Benefício da Terapêutica Treino de Exercício de Exercício e Ressincronização Cardíaca em doentes com Insuficiência Cardíaca
(BETTER-HF)

1. RESUMO

Introdução

A insuficiência cardíaca crónica é conhecida como síndrome complexa, associada a elevada mortalidade e incapacidade, envolvendo múltiplos mecanismos fisiopatológicos, neuro-hormonais, endoteliais e inflamatórios.

Além da terapêutica médica optimizada, a terapêutica não farmacológica, como a ressincronização cardíaca e o treino de exercício, assume um papel fundamental.

Na insuficiência cardíaca avançada, doentes com critérios para terapêutica de ressincronização cardíaca (CRT) têm sido exaustivamente estudados, apesar da maioria dos estudos não se ter dedicado à diversidade de efeitos e mecanismos fisiopatológicos envolvidos, nos doentes mais gravemente sintomáticos.

Nesta população com insuficiência cardíaca avançada tratada com CRT, estudos relativos aos efeitos e mecanismos do treino de exercício, especificamente exercício intervalado de alta intensidade, são ainda poucos e de pequena dimensão.

Hipótese

Hpótese principal formulada:

Existe benefício em associar um programa de treino de exercício de alta intensidade, de longa duração, após ressincronização cardíaca em doentes com insuficiência cardíaca avançada.

Hipótese secundária:

Estão envolvidos vários mecanismos fisiopatológicos, contribuindo diferentemente para o benefício do treino de exercício após CRT e para o benefício de CRT sem programa de exercício subsequente, em doentes com insuficiência cardíaca avançada.

Objectivos

O objectivo primário desta tese foi determinar os efeitos do programa de exercício intervalado de alta intensidade (HIIT), de longa duração, sobre a classe funcional clínica, qualidade de vida, capacidade funcional de exercício, função cardíaca e remodelagem ventricular, em doentes com insuficiência cardíaca avançada após implante do ressincronizador.

O objectivo secundário pretendeu avaliar o papel potencial de diferentes mecanismos fisiopatológicos nos benefícios do treino de exercício após CRT, HIIT, e após CRT sem exercício subsequente: função endotelial, função do sistema nervoso autónomo, processo inflamatório e apoptose.

Metodologia

Efectuámos um ensaio controlado aleatorizado para determinar os efeitos da intervenção de exercício, HIIT, em doentes com insuficiência cardíaca avançada após CRT.

Os critérios de inclusão foram, doentes com insuficiência cardíaca estável, em classe III-IV (NYHA), sob terapêutica farmacológica optimizada, referenciados para CRT pelas recomendações actuais presentes, etiologia isquémica e não isquémica, com idade superior a 18 anos. Os critérios de exclusão incluiram insuficiência cardíaca instável, doença ortopédica ou muscular incapacitante para exercício e residência geograficamente distante do hospital.

Os doentes que preencheram os critérios de inclusão foram aleatorizados para treino de exercício intervalado de alta intensidade ou para grupo controlo (EXTG e CG, respectivamente).

A aleatorização, realizada por um investigador independente, foi estratificada, baseada na idade (<65 ou ≥65 anos), sexo, etiologia (isquémica e não isquémica) e gravidade de disfunção ventricular esquerda (fracção de ejecção ventricular esquerda <20 ou ≥20%).

Os doentes com os mesmos critérios de inclusão, que não aceitaram a intervenção exercício ou que viviam longe, sem os restantes critérios de exclusão, foram adicionalmente estudados como cohort prospectivo para avaliação dos efeitos e mecanismos da intervenção CRT.

Durante o periodo de Janeiro 2012 a Março 2015, todos os doentes com insuficiência cardíaca e critérios para ressincronização cardíaca elegíveis foram estudados.

O programa de treino de exercício foi iniciado 1 mês após implante de cardioressincronizador e durou 6 meses com frequência bissemanal, consistindo em sessões de 60 minutos, realizadas no hospital, monitorizadas e supervisionadas. Incluiu treino aeróbio intervalado de alta intensidade (HIIT), adaptado a partir do protocolo de Wisloff, e exercícios de resistência, flexibilidade e coordenação.

Os momentos do estudo usados para avaliação das variáveis independentes foram: momento basal, pré implante do ressincronizador (M1), aos 3 meses após exercício, correspondendo a 4 meses após implante (M2) e aos 6 meses após exercício, correspondendo a 7 meses após implante (M3).

As variáveis dependentes estudadas foram: classe functional clínica (NYHA), scores de qualidade de vida (questionário HeartQol), parâmetros de função cardíaca e remodelagem reversa (determinadas por ecocardiografia e doseamento plasmático de péptido natriurético, BNP), de capacidade funcional de exercício (determinadas por prova de esforço cardio-respiratória, CPT), de função do sistema nervoso autonómico, SNA (por cintigrafia cardíaca com ^{123}I-MIBG, prova de esforço cardio-respiratória e análise da variabilidade da frequência cardíaca no Holter-24 horas), de função endotelial e rigidez arterial (determinada por doseamento de NO, óxido nítrico, e por PAT, tonometria arterial periférica), marcadores de inflamação e apoptose (medição de proteína C reactiva de alta sensibilidade, hs-CPR, factor de necrose tumoral alfa, TNF-α, interleucina-6, IL-6, fracção solúvel do cluster de diferenciação 40, sCD40, fracção solúvel do ligando Fas, sFasL) e frequência de eventos major cardiovasculares aos 6 meses de exercício.

As excepções aos 3 momentos de avaliação foram: ^{123}I-MIBG cintigrafia cardíaca, realizada antes do CRT (M1) e aos 6 meses de exercício (M3), análise de variabilidade da frequência cardíaca por estudo Holter-24horas, realizado apenas basal, pre-CRT (M1) e frequência de eventos, avaliada em M3.

A segurança do treino de exercício HIIT foi avaliada.

A resposta ecocardiográfica foi definida pelo aumento de pelo menos 5% da fracção de ejecção ventricular esquerda (LVEF), em valor absoluto e a resposta clínica como melhoria de pelo menos 1 classe funcional clínica (NYHA). A resposta funcional foi definida como o aumento de pelo menos 1 ml/kg/min de VO_2p.

Resultados

A partir de um cohort inicial de 121 doentes com insuficiência cardíaca selecionados para CRT, foram aleatorizados 62 doentes. Realizaram programa de treino de exercício HIIT, 22 doentes (EXTG), idade média 67,5±9,8%, 22,7% do sexo feminino, 40% isquémicos, LVEF basal 26,68±6,21%, enquanto 28 doentes foram incluídos no grupo controlo (CG). As características demográficas e clínicas basais foram idênticas estatisticamente.

No grupo aleatorizado (n=50), todos os doentes tiveram benefício significativo, aos 6 meses após início do exercício, relativamente a: diminuição da classe clínica de NYHA ($p < 0,001$), melhoria do score de qualidade de vida HeartQol ($p < 0,001$), aumento da LVEF, fracção de ejecção ventricular esquerda ($p < 0,005$), diminuição dos volumes ventriculares esquerdos, LVED, tele-diastólico ($p < 0,05$) e LVES, tele-sistólico ($p < 0,02$). Verificou-se uma diferença significativa da classe funcional clínica (NYHA), nos dois grupos aleatorizados, com maior diminuição no EXTG ($p=0,034$). Apenas no EXTG, se encontrou um aumento significativo da duração da prova de esforço cardio-respiratória, aos 3 meses ($p=0,017$) e aos 6 meses ($p=0,008$). O tempo para o limiar anaeróbio, VAT, aumentou significativamente no EXTG aos 3 meses ($p= 0,006$) e aos 6 meses ($p=0,004$), sendo significativamente diferente do CG aos 3 meses ($p=0,006$) e apresentando uma tendência para significado estatístico aos 6 meses ($p=0,064$), momento em que a variação foi também significativa no CG. O TNF-α diminuiu significativamente apenas no EXTG, aos 6 meses ($p=0,016$), com uma diferença estatística significativa em relação ao

CG (p=0,008). Não se verificaram diferenças significativas nas variações dos parâmetros ecocardiográficos entre os dois grupos aleatorizados. Relativamente ao número de respondedores, no grupo de treino de exercício foram identificados mais respondedores clínicos (95%) e ecocardiográficos (81,8%) que no grupo controlo (78,5% e 72,7%, respectivamente), após 6 meses de exercício. A diferença no número de respondedores entre os 2 grupos aleatorizados, não atingiu contudo significado estatístico (provavelmente pela dimensão da amostra), mas com uma tendência para mais respondedores clínicos no grupo de exercício. A diferença no numero de respondedores funcionais, apesar de em numero tendencialmente superior no grupo de exercício (77,2%) não foi significativa. O programa HIIT mostrou ser seguro, sem eventos major ou minor durante o exercício. Aos 6 meses de exercício (7 meses após implantação do ressincronizador), registaram-se 9% de eventos no grupo exercício e 10,7% no grupo controlo. Verificou-se ocorrência de morte ou internamento hospitalar em 1/22 doentes (4,5%) do grupo de exercício e em 3/28 doentes (10,7%) do grupo controlo. A única morte nos doentes aleatorizados ocorreu no grupo controlo, 1/28 doentes (3,5%).

No total do cohort de doentes com CRT verificou-se um benefício significativo após 7 meses de implantação: redução da classe funcional NYHA (p <0,001), aumento do score HeartQol (p <0,001), aumento da LVEF (p < 0,001), diminuição do volume tele-sistólico ventricular esquerdo (p=0,001), aumento do valor absoluto de GLS, strain global longitudinal, (p=0,003), relação E/e', rácio entre onda E do fluxo de câmara de entrada do ventrículo esquerdo e e' médio de doppler tecidular do anel mitral, (p=0,009), redução da massa ventricular esquerda (p=0,026), redução do VE/VCO_2 slope, declive da razão entre ventilação minuto e produção de CO_2, (p=0,003), aumento da duração do teste cardiopulmonar (p=0,002), aumento do tempo para VAT, limiar anaeróbio (p=0,001), redução do HRR1 (frequência cardíaca de recuperação ao primeiro minuto), (p=0,015), redução do HRR6 (frequência cardíaca de recuperação ao 6º minuto), (p=0,033) e aumento do $VO_{2p,}$ consumo de oxigénio pico, (p=0,04). Na amostra total dos doentes insuficientes cardíacos com CRT (incluindo 18% dos doentes submetidos a exercício) 75,6% foram respondedores clínicos, 63,9% respondedores ecocardiográficos e 62,8% respondedores funcionais. Os respondedores ecocardiográficos ao CRT tinham diferenças significativas nos parâmetros de base e na variação de alguns parâmetros: M1, menores volumes ventriculares esquerdos, maior TAPSE, maior SDNN (standard

deviation NN interval), maior heart-mediastinum ratio precoce (HMRe) e tardio (HMRl); M3-M1, maior aumento de LVEF, maior redução de volume LVES, maior aumento do valor absoluto de GLS e tendência para maior aumento de VO2p. Os respondedores tiveram menor número de eventos major registados em M3.

Analizando todos os doentes com CRT, valores de ^{123}MIBG HMRl>1,5 identificaram mais respondedores ecocardiográficos (probabilidade 2 vezes superior), apenas em não isquemicos.

Os eventos aos 7 meses após CRT, M3, morte ou admissão hospitalar ou arritmia ocorreram em 14,8% da população total e em 16,1% dos doentes não submetidos a exercício.A morte ocorreu em 4,9% no grupo total e em 6% do grupo não submetido a exercício.

Conclusão

No presente ensaio aleatorizado e controlado, realizado numa amostra de doentes com insuficiência cardíaca avançada, referenciada para CRT, o exercício HIIT após implante do ressincronizador provou ser benéfico e seguro, associado a um maior número de respondedores ecocardiográficos e clínicos, acompanhado de uma melhoria clínica mais significativa, evidenciando o benefício adicional ao CRT. A melhoria do componente periférico da insuficiência cardíaca condicionada pelo exercício foi demonstrada pelo aumento significativo da capacidade funcional ao esforço e do tempo para VAT, acompanhada de maior número de respondedores funcionais, tendo-se verificado um efeito modulatório sobre a inflamação que poderá ter contribuído para este efeito. Não foram demonstrados benefícios do exercício na função endotelial, no sistema nervoso autonómico e na apoptose. Ocorreram menos eventos major aos 6 meses em doentes submetidos a HIIT.

A avaliação adicional dos doentes com CRT no estudo observacional demonstrou melhoria clínica, de qualidade de vida e de função ventricular sistólica e diastólica significativa, mesmo excluindo aqueles que fizeram treino de exercício. O efeito central do CRT na remodelagem cardíaca demonstrou ser crucial, com melhoria das diversas variáveis ecocardiográficas. Contrariamente, não se demonstraram efeitos periféricos benéficos do CRT, VO2p, duração CPT ou tempo VAT, aos 7 meses, uma vez excluídos os

doentes que fizeram programa de exercício. O sistema nervoso autónomo demonstrou ser um mecanismo relevante na resposta ao CRT, mas apenas em insuficientes cardíacos não isquémicos. Não foram demonstrados efeitos benéficos do CRT na função endotelial, inflamação ou apoptose. Registaram-se mais eventos em doentes sem terapêutica de exercício.

Dos resultados desta tese, que verificam as hipóteses colocadas, podemos salientar que em doentes com insuficiência cardíaca avançada a intervenção de treino de exercício intervalado de alta intensidade, supervisionado, , após implantação de ressincronizador cardíaco é uma terapêutica não farmacológica segura e tem benefício adicional demonstrado relativo à CRT, resultando em menor número de doentes não respondedores. Esta intervenção não teve efeito deletério sobre a remodelagem reversa e alguns resultados apontam para potencial benefício. Os mecanismos envolvidos estão ligados particularmente ao componente periférico da insuficiência cardíaca, resultando em diminuição da gravidade dos sintomas clínicos, melhoria da capacidade funcional e modulação positiva da resposta fisiopatológica inflamatória.

2. SUMMARY

Introduction

Chronic heart failure is known to be a complex syndrome, associated to high mortality and disability, involving multiple pathophysiologic mechanisms, neuro-hormonal, endothelial and inflammatory.

Besides optimized medication, the nonpharmacologic therapy, like cardiac resynchronization and exercise training, plays a fundamental role.

In advanced heart failure, patients with criteria for cardiac resynchronization therapy (CRT) have been studied extensively, though most of the studies were not dedicated to the diversity of effects and involved pathophysiologic mechanisms, in most severely symptomatic patients.

In this advanced heart failure population treated with CRT, studies regarding exercise training effects and mechanisms, specifically high intensity interval exercise, are still few and small-sized.

Hypothesis

Main hypothesis formulated:

It is beneficial to associate a high intensity interval training exercise program, long duration, after cardiac resynchronization in advanced Heart Failure Patients.

Secondary hypothesis:

Several pathophysiologic mechanisms are involved, contributing differently to the exercise training benefit after CRT and to the benefit of CRT without subsequent exercise program in advanced HF patients.

Aims

The primary aim of this thesis was to determine the effects of a long-term High Intensity Interval Exercise Training (HIIT) program on clinical functional class, quality of life, exercise functional capacity, cardiac function and remodeling, in advanced heart failure patients after cardiac resynchronizer implant.

Secondary aim intends to evaluate the potential role of different pathophysiologic mechanisms in the benefits of exercise training after CRT, HIIT, and of CRT without subsequent exercise: endothelial function, autonomic nervous system function, inflammatory process and apoptosis.

Methodology

A randomized controlled trial was performed to determine the effects of exercise intervention, HIIT, in advanced heart failure patients after CRT.

The inclusion criteria considered patients with stable heart failure, class III-IV (NYHA), receiving optimal pharmacologic therapy, assigned to CRT by present guidelines, ischemic and non ischemic etiology, older than 18 years. Exclusion criteria included unstable HF patients, exercise incapacitating orthopedic or muscular disease and geographically long distance living.

Patients who fulfilled the inclusion criteria were randomized for long duration high intensity interval exercise training or for control group (EXTG and CG, respectively).

Randomization, performed by an independent investigator, was stratified, based on age (<or≥65 years), gender, etiology (ischemic and non ischemic) and severity of left ventricular dysfunction (left ventricular ejection fraction <20% or ≥20%).

Patients with the same inclusion criteria, who did not accept exercise intervention or living far, without other exclusion criteria were additionally studied as a prospective cohort for evaluation of CRT intervention effects and mechanisms.

During the period from January 2012 to March 2015, all patients with chronic heart failure and criteria for cardiac resynchronization were evaluated.

The exercise training program started 1 month after cardiac resynchronizer implant and lasted 6 months, twice a week, consisting of 60 minutes hospital-based, monitored, supervised sessions, starting at 1 month after CRT onset. It included aerobic high intensity interval training (HIIT), adapted from Wisloff protocol, and exercises of resistance, flexibility and coordination.

Moments of the study used for the evaluation of independent variables were baseline, before cardioresynchronizer implant (M1), at 3 months of exercise, corresponding to 4 months after CRT (M2) and at 6 months (M3) after exercise, corresponding to 4 months (M2) and 7 months, corresponding to 7 months after CRT (M3).

Dependent variables studied were: clinical functional class (NYHA), quality of life scores (HeartQol questionnaire), parameters of cardiac function and reverse remodeling (determined by echocardiography and BNP, plasmatic brain natriuretic peptide, measurement), of functional exercise capacity (determined by cardiopulmonary exercise testing, CPT), of autonomic nervous system function, ANS (determined by ^{123}I-MIBG cardiac scintigraphy, cardiopulmonary exercise testing and 24-hours-holter heart rate variability analysis), of endothelial function and arterial stiffness (determined by NO, plasmatic Nitric Oxide measurement and PAT, peripheral arterial tonometry), inflammation and apoptosis (by measurement of high sensitivity C reactive protein, hs-CPR, Tumor Necrosis Factor alpha, TNF-α, Interleukin-6, IL-6, soluble cluster of differentiation 40, sCD40, soluble ligand of Fas, sFasL) and frequency of major cardiac events identification at 6 months of exercise.

Exceptions to the 3 moments were, ^{123}I-MIBG cardiac scintigraphy, which was performed before CRT (M1) and at 6 months after exercise (M3), 24hours-holter heart rate variability study, performed only baseline, pre-CRT (M1) and cardiac events evaluation at M3.

The safety of HIIT exercise was evaluated.

CRT echocardiographic response was defined by the increase of at least 5% of left ventricular ejection fraction (absolute value) and clinical response was defined as the improvement of at least 1 clinical functional class (NYHA). Functional response was defined as the increase of at least 1 mg/kg/min VO_2p.

Results

From the initial cohort sample of 121 heart failure patients selected for CRT, 62 patients were ramdomized. Exercise training program HIIT was performed by 22 patients (EXTG), mean age 67.5±9.8 years old, 22.7% female, 40% ischemic, baseline LVEF 26.68±6.21%, while 28 patients were assigned to the control group (CG). Demographic and baseline clinical characteristics were statistically identical.

In the randomized sample (n=50), all patients had significant benefit, at 6 months after exercise onset (M3), regarding: NYHA, New York Heart Association, decrease ($p< 0.001$), HeartQol score improvement ($p<0.001$), LVEF, left ventricular ejection fraction increase ($p<0.005$), LVED, left ventricular end-diastolic volume ($p< 0.05$) and LVES, left ventricular end-systolic volume decrease ($p<0.02$). There was a significant difference in the decrease of clinical functional class of NYHA in the two randomized groups, greater in EXTG ($p=0.034$).

Only in EXTG, there was a significant CPT (cardiopulmonary testing) duration increase at 3 months ($p=0.017$) and at 6 months ($p=0.008$). VATtime (time to ventilatory anaerobic threshold) significantly increased in EXTG at 3 months ($p=0.006$) and at 6 months ($p=0.004$), being significantly different regarding the CG at 3 months ($p=0.006$) and showing a tendency to statistical significance at 6 months ($p=0.064$), when variation was also significant in CG. TNF-α decreased significantly, only in EXTG, at 6 months ($p=0.016$) with a statistical difference from CG ($p=0.008$). There were no significant differences in echocardiographic parameters between the two randomized groups. Regarding the

number of CRT responders, in the exercise group, there were more CRT clinical (95%) , echocardiographic (81.8%) and functional (77.2%) responders than in the control group (78.5%, 72.7% and 53.8%, respectively), after 6 months of exercise. The difference in the number of responders in the two randomized groups, however, did not reach statistical significance (probably because of the sample size), but revealed a tendency for greater number of clinical and functional responders in the exercise group.

HIIT program turned out to be safe, without any major or minor events during exercise. At 6 months after exercise (7 months after CRT device implant), death or hospital cardiac admission occurred in 1/22 patients (4.5%) of the exercise group and in 3/28 patients (10.7%) of the control group. The only death in the randomized patients occurred in the control group, 1/28 patients (3.5%).

In the total CRT patients cohort there was a significant benefit after cardiac resynchronizer implant, at 7 months: functional NYHA decrease ($p<0.001$), HeartQol score increase ($p<0.001$), LVEF increase ($p<0.001$), LVES volume decrease ($p=0.001$), GLS (left ventricular global longitudinal strain) absolute value increase ($p=0.003$), E/e' (ratio between E wave from pulsed Doppler left ventricular inflow wave and tissue Doppler mitral annular mean e' decrease ($p=0.009$), LVM, left ventricular mass decrease ($p=0.026$), VE/VCO_2 slope, minute ventilation to carbon dioxide production ratio slope decrease ($p=0.003$), cardiopulmonary testing duration increase ($p=0.002$), VATtime increase ($p=0.001$), HRR1, Heart Rate Recovery at 1^{st} minute decrease ($p=0.015$), HRR6, Heart rate recovery at 6^{th} minutes decrease ($p=0.033$) and $VO_{2p,}$ peak oxygen consumption increase ($p=0.04$).

In total HF patients sample, after CRT (including 18% of the patients submitted to exercise), 75.6% were clinical responders, 63.9% were echocardiographic responders and 62.8% were functional responders.

CRT echocardiographic responders had significant differences in baseline parameters and in the variation of some parameters: M1, smaller left ventricular volumes, greater TAPSE, greater SDNN (standard deviation NN interval), greater heart-mediastinum ratio, early (HMRe) and late (HMRl); M3-M1, greater increase of LVEF, greater reduction of LVES volume, greater increase in GLS absolute value and tendency for greater increase in VO2p. Responders had less major events registered at M3.

Analyzing the total HF-CRT patients, values of HMR late>1.5 identified more CRT echocardiographic responders (2-fold probability), only in nonischemic.

Events at 7 months after CRT, M3, cardiac death or hospital admission or arrhythmia occurred in 14.8% of total population and in 16.2% of nonrandomized patients. Death occurred in 4.9% in total group and in 6% in nonrandomized group.

Conclusion

In this controlled randomized trial, performed in a sample of advanced HF patients referred to CRT, HIIT exercise after cardiac resynchronizer implant proved to be beneficial and safe, associated to an increased number of clinical and echocardiographic responders and with more significant clinical improvement, suggesting an additional benefit to CRT. The improvement of the peripheral component of heart failure caused by exercise was demonstrated by CPT duration and time to VAT significant increase, associated with more functional responders, along with positive modulation of inflammation, which might have contributed to this effect. No significant effects were demonstrated in endothelial or autonomic nervous system function. Less major events occurred in the HIIT group after the 6 months of training.

The additional evaluation of CRT patients in the observational study of the total HF sample, showed a beneficial effect on symptoms severity, quality of life and systolic and diastolic LV function, even excluding those who performed exercise. Central effect of CRT on cardiac remodeling demonstrated to be crucial, with echocardiographic improvement of several variables. Once EXTG patients were excluded, the restant CRT patients did not show significant improvement at 7 months of VO_{2p}, CPT duration or time to VAT, meaning CRT had no effect on HF peripheral component. Autonomic nervous system demonstrated to be a relevant mechanism for CRT response, but only in nonischemic HF.

No beneficial effects of CRT were noticed in endothelial function, inflammation or apoptosis. More events were registered in patients who did not exercise.

From these thesis results, we may accept, in advanced heart failure patients, exercise (HIIT) as safe and beneficial nonpharmacologic therapy with demonstrated additional benefit, regarding CRT, resulting in fewer patients with CRT nonresponse. This

intervention had no deleterious effect on reverse remodeling and some results point out to a potential benefit. The involved mechanism especially regards the peripheral component of HF, manifested by the decrease in clinical symptoms severity, improvement in functional capacity and positive modulation of pathophysiologic inflammatory response. (FCT PTDC/DES/120249/2010)

3. INTRODUCTION

Chronic heart failure (HF) is known for long as a clinical syndrome, including reduced exercise tolerance (dyspnea and/or fatigue), which is based on complex pathophysiology. It is usually characterized by left ventricular (LV) systolic dysfunction leading to systemic and pulmonary congestion and elevated peripheral vascular resistance, though it may occur with preserved LV ejection fraction, HFpEF[1]. Several pathophysiologic mechanisms are involved and play an important role in HF negative progression. Optimized pharmacologic therapy, intervening on these mechanisms, led to a decrease in mortality[2].

Non pharmacologic therapy, including devices, has evolved more recently.

Cardiac resynchronization therapy (CRT) manages to improve HF patient's prognosis[3.4]. Besides decreasing mortality[4], it has improved symptoms and left ventricular function (LV) in most patients[5], however at least 30% of the patients do not respond to this therapy, as demonstrated in all major trials[6].

CRT responders show a significant LV end-systolic volume (LVESVol) decrease, LV ejection fraction (LVEF) and 6-minute walk test (6MWT) increase and improvemement of clinical functional class (NYHA), quality of life (QOL) and endothelial function[5,7,8]. Identifying the nonresponders, at higher risk of death, and those who most likely may benefit from currently available therapeutic technologies, remains a challenge.

In the last 20 years, there has been a growing consensus on exercise training (EXT) beneficial effects in HF patients[9,10]. The benefits of moderate EXT have been demonstrated by significant improvements in exercise capacity, quality of life (QOL) and reduction of hospitalizations in HF patients[11,16], with rare and minor adverse events, during the training and after in the follow-up[17,18].

The rationale of EXT in patients with internal cardiac defibrillator (ICD) and CRT is based on its favorable documented effects on functional capacity, autonomic balance, myocardial perfusion and LV function, already described in stable HF patients[19].

It has also been demonstrated, in ICD and in CRT patients that, besides beneficial effects on functional capacity associated to quality of life and outcome, EXT is safe[20].

EXT corrects most of the peripheral abnormalities encountered in HF and decreases neurohormonal stimulation without a deleterious effect on LV remodeling[10]. As we are aware, abnormalities in endothelium- and flow-depend vasodilatation are a key factor in mortality and morbidity of patients with depressed LV ejection fraction

(LVEF) and prolonged QRS, who are moderate to severely symptomatic, despite optimal medical therapy. EXT enables the improvement of both basal endothelial nitric oxide formation (NO) and agonist-mediated endothelial-dependent vasodilatation of the skeletal muscle vasculature in HF patients. The correction of endothelial dysfunction (endothelium-dependent change in peripheral blood flow) was associated with a significant improvement in exercise capacity evidenced by peak oxygen uptake increase[21,22]. These findings are of major importance, once HF patients with the greatest sympathetic activation and the most reduced endothelial function will have the poorest prognosis and are in most need for intervention.

Importantly, it has to be noticed that most of the studies of EXT performed in HF have been conducted in patients without severe functional impairment. Very little information is currently available on patients in NYHA class III-IV and these are the ones who mostly will require ICD/CRT and may need additional intervention, like exercise training, especially if they turned to be CRT nonresponders.

It is not well known how HF patients, with more severe functional limitation, respond to EXT and, more importantly, what are the physiologic mechanisms and how can they explain the improvements in HF, as a consequence of EXT.

This lack of scientific information is urgent to fulfill, since this is the group of patients (NYHA class III-IV) who normally is targeted for CRT.

It is not clearly established if, adding an exercise training (EXT) program after cardiac resynchronizer implant, provides better clinical outcome than CRT alone.

Prior studies on CRT and EXT, preliminary in nature, employed only 3-months EXT programs, suggesting small improvements in functional capacity[23], although not providing information on potential mechanisms.

We proposed, in this thesis, to evaluate in a population of advanced HF on optimized pharmacologic therapy, the effects and subjacent mechanisms of nonpharmacologic intervention: a program of long duration (6 months) of high intensity interval training after CRT (HIIT), in comparison with cardiac resynchronization therapy alone (CRT) .

The study intended mainly to determine whether a long-term high intensity interval exercise training program provides additional benefits, with better clinical outcomes, than CRT alone, namely in CRT nonresponders, and to identify the mechanisms of the hypothetized improvement.

Understanding the potential mechanisms associated with clinical, echocardiographic, and functional improvements is essential to ameliorate the rehabilitative process and develop new innovative therapies in this high risk population.

4. BACKGROUND

4.1. Heart failure and pathophysiologic mechanisms

Heart failure (HF) is defined as a clinical syndrome that develops in consequence to a cardiac injury, causing decline in heart function, systolic or diastolic.

Many definitions of HF have been put over the last years[24], highlighting one or several features of this complex syndrome[24-27], such as hemodynamics, oxygen consumption or exercise capacity. In recent years, most definitions have emphasized the need for both the presence of symptoms, dyspnea and fatigue, which may limit exercise tolerance, and physical signs of fluid retention, peripheral edema and/or pulmonary and/or splanchnic congestion. Because not all the patients present symptoms or signs of volume overload, the term "heart failure" is preferred over "congestive heart failure"[25].

Multiple etiologies are behind HF, including coronary artery disease, hypertension, myocarditis, valve or congenital disease, not always identified, like in idiopathic cardiomyopathy[25].

The prevalence of the HF syndrome has significantly increased during the last years, remaining a substantial health burden[28]. Heart failure is the leading cause of hospitalization in people aged 65 years or older[29,30] and associates to other negative outcomes, including disability, poor quality of life, polipharmacy side effects and increase in morbidity and mortality [31,32]. Over the past 50 years, survival after HF onset has improved, probably due to more effective treatment of hypertension, coronary artery disease, valve disease and to the increasing use of pharmacologic therapies[33]. Also, HF annual mortality has been reduced due to HF optimized pharmacologic therapy[34].

A complex pathophysiology underlies, usually characterized by left ventricular (LV) dysfunction (more frequently systolic), resulting from any structural or functional impairment of ventricular filling or ejection of blood and leading to systemic and pulmonary congestion and elevated peripheral vascular resistance[35]. From a pathophysiologic point of view, HF is characterized by a continuous interplay between the underlying myocardial dysfunction and the compensatory neurohumoral

mechanisms, which are initially able to compensate for the decreased myocardial function, supporting cardiac output, in response to the reduced heart function, and preserving cardiovascular equilibrium. Contrarily, at long-term, these exert deleterious effects on cardiac structure and performance, which lead to cardiac decompensation and progressive aggravation of left ventricular dysfunction. Progressive left ventricular remodeling and left ventricular dysfunction become at some moment self-sustained[36].

The main neurohumoral mechanisms involved consist of elevated activities of the adrenergic (or sympathetic) autonomic nervous system (ANS), of the renin–angiotensin–aldosterone system (RAAS) and of several cytokines[35].

Autonomic Nervous System

Autonomic Nervous System function

Most of the data about the role of ANS in the development and prognosis of HF were obtained from studies with dilated ventricles and reduced LVEF[37].

Activation of ANS, probably the most proeminent neurohumoral mechanism of HF, is the first response to myocardial injury or to changes in cardiac loading[37]. It is responsible for a wide variety of cardiovascular effects: positive chronotropy (heart rate acceleration, arrhythmia predisposition), positive inotropy (increase in cardiac contractility), positive lusitropy (accelerated cardiac relaxation), venous capacitance decrease, resistance and cutaneous vessels constriction. These effects, intend to improve cardiac performance, preparing the body for *fight-or-flight response*[36].

Cardiovascular ANS activation leads to the release of norepinephrine (NE) and epinephrine, the neurotransmitters that mediate its effects, by several mechanisms[36]:

1-Norepinephrine release by cardiac sympathetic nerve terminals located in the right stellate ganglion reach the sinus and atrioventricular nodes (increase in heart rate and shortening of atrioventricular conduction) and in the left stellate ganglion reach the left ventricle (increase in contractile strength), although norepinephrine release and reuptake can occur throughout the heart;

2- Epinephrine (and norepinephrine to much lesser extent) release into the circulation by the adrenal medulla, affecting both the myocardium and peripheral vessels;

3- Norepinephrine and epinephrine local release by various autonomic peripheral nerve terminals, which can synthesize and release these catecholamines in an autocrine/paracrine manner, located in blood vessels and in cardiac myocytes themselves[38,39].

Norepinephrine and epinephrine, mediate their effects in cells and tissues by binding to specific cell surface adrenergic receptors (ARs), which belong to the superfamily of G-protein-coupled receptors (GPCRs), to the 7 transmembrane-spanning receptors or to heptahelical receptors. The norepinephrine transporter type 1 recycles approximately 80% of norepinephrine released by autonomic nerve terminals, whereas the remainder spills over into the circulation[40].

The receptors for both ANS catecholamines (AR) are divided into 3 types and 9 total different subtypes[41], as follows:

- 3 α1AR subtypes - α1A, α1B, α1D
- 3 α2AR subtypes - α2A, α2B, α2C
- 3 βAR subtypes - β1, β2, β3

All AR primarily signal through heterotrimeric G proteins. The human heart contains all 3 βAR subtypes[42].

In the healthy myocardium β1AR is the predominant subtype, representing 75% to 80% of total βAR density, followed by β2AR, which comprises ≈15% to 18% of total cardiomyocyte βARs, and the remaining 2% to 3% is β3ARs[43].

The principal role of βARs in the heart is the regulation of cardiac rate and contractility in response to norepinephrine and epinephrine[43].

Stimulation of β1ARs (mainly) and stimulation of β2ARs (to a lesser extent) increase cardiac contractility (positive inotropic effect), frequency (positive chronotropic effect), and rate of relaxation (lusitropic effect), as well as accelerates impulse conduction through the atrioventricular node (positive dromotropic effect) and pacemaker activity from the sinoatrial node[44].

β3ARs are predominantly inactive during normal physiological conditions[45], however, their stimulation seems to produce a negative inotropic effect, opposite to that induced by β1ARs and β2ARs, involving the nitric oxide synthase pathway[46], thus acting as a fuse against cardiac adrenergic overstimulation[47].

Agonist-induced activation of βARs catalyzes the exchange of GTP for GDP on the Gα subunit of heterotrimeric G proteins, resulting in the dissociation of the heterotrimer into active Gα and free Gβγ subunits (always associated together, a heterodimer that functions essentially as a monomer), which can transduce intracellular signals independently of each other[48]. The most powerful physiological mechanism to increase cardiac performance is activation of cardiomyocyte β1ARs and β2ARs, which, in turn, activate Gs proteins (stimulatory G proteins). Gs-protein signaling stimulates the effector adenylate cyclase, that converts ATP to the second messenger adenosine 3′,5′-monophosphate or cAMP, which in turn binds to and activates the cAMP-dependent protein kinase (protein kinase A[PKA]). PKA is the major effector of cAMP (there is also Epac, exchange protein directly activated by cAMP, which can be activated by cAMP independently of PKA), and by phosphorylating a variety of substrates, it ultimately results in significant increase in free intracellular Ca2+ concentration, which is the master regulator of cardiac muscle contraction[49].

There are some important genetic polymorphisms in human βAR and αAR genes, which were associated with HF phenotypes and interaction with β-blocker therapy (a mainstay of HF standard of care) and can significantly influence cardiac function[50]. AR genetic polymorphisms may prove to be useful tools in guiding the individual tailoring of HF therapy in the future[51-53].

The delicate balance between hyperstimulation and hypostimulation of the above mentioned 9 types of adrenergic receptors in the various forms and stages of HF remains to be explored. Additional agonists and antagonists that are specific for each of these receptors remain to be discovered[54].

ANS hyperactivity is evidenced by increased plasma norepinephrine and epinephrine levels, elevated (central) sympathetic outflow, and heightened norepinephrine spillover from activated cardiac sympathetic nerve terminals into the circulation[55]. Cardiac norepinephrine spillover in untreated HF patients can reach until 50-fold higher levels than those of healthy individuals under maximal exercise conditions[56].

In HF with preserved left ventricular ejection fraction, the information on chronic ANS activation is limited. ANS hyperactivity in patients with hypertension may contribute to the development of left ventricular diastolic dysfunction and thus increase HF risk[57].

In systolic HF, patients actually may have ANS neuronal density and function decreased, resulting in diminished norepinephrine concentration within the heart, in addition to decreased postsynaptic βAR density, due to depletion of cardiac ANS neuronal norepinephrine storage and decreased norepinephrine presynaptic reuptake secondary to norepinephrine transporter downregulation[58,59].

The elevated ANS outflow and norepinephrine and epinephrine levels in chronic HF lead to chronically elevated stimulation of the cardiac βAR system, which has detrimental repercussions for the failing heart.

Cardiac βAR dysfunction in human HF is characterized at the molecular level by selective reduction of β1AR density at the plasma membrane (downregulation) and by uncoupling of the remaining membrane β1ARs and β2ARs from G proteins (functional desensitization)[60]. Importantly, myocardial levels and activities of the most important, versatile, and ubiquitous GRKs, GRK2 and GRK5, are elevated both in humans and in animal models of HF[61-65].

The current consensus is that in chronic HF the excessive amount of ANS, derived catecholamines hitting cardiac βARs extracellularly, triggers the GRK2 upregulation inside the cardiomyocytes, thus leading to a reduction in cardiac βAR density and responsiveness and resulting in cardiac inotropic reserve depletion[66,67]. This GRK2 elevation possibly serves as a homeostatic protective mechanism aimed at defending the heart against excessive catecholaminergic toxicity. However, several studies refuted this assumption, demonstrating that GRK2 upregulation is detrimental for the heart and causes the functional uncoupling of βARs *in vivo*[65]. This finding prompted investigations of the role GRK2 plays in cardiac function, which revealed that cardiac GRK2 is an absolutely critical regulator of cardiac βAR–dependent contractility and function. Specifically, cardiomyocyte-restricted overexpression of GRK2 to the same level of upregulation found in human HF (ie, 3-fold to 4-fold) markedly attenuated βAR signaling and contractile reserve, showing that GRK2 is the main culprit for the functional desensitization of cardiac βARs in HF[68]. On the down side, loss of cardiac GRK2 can predispose the heart to catecholamine toxicity, exactly because it works, in essence, as a positive inotropic therapy[69].

In summary, the elevated ANS activity in chronic HF causes enhanced GRK2–mediated cardiac β1AR and β2AR desensitization and β1AR down regulation, which leads to the

progressive loss of the adrenergic and inotropic reserves of the heart, the molecular abnormality hallmark of this disease[70]. With regard to the other major AR type expressed in the heart, α1ARs in HF may function in a compensatory fashion to maintain cardiac inotropy, but their involvement in cardiac pathophysiology appears limited to situations of cardiac hypertrophy that ultimately lead to HF[71]. In the presence of pressure overload, cardiac α1AARs get activated and promote cardiomyocyte survival, blocking apoptosis and protecting against adverse remodeling and decompensation to HF [72,73].

Imaging of Autonomic Nervous System

A noninvasive imaging tool, that directly assesses cardiac sympathetic neuronal activity, is ^{123}I-MIBG cardiac scintigraphy. This technique, not only displays the presence of noradrenergic innervation, but also its functional capability[74-76].

^{123}I-MIBG is a radio-labelled analogue of the potent neuron-blocking agent guanethidine, that acts selectively on sympathetic nerve endings. Uptake of ^{123}I-MIBG into the neurons is achieved mainly through the uptake-1 mechanism, an homeostatic system responsible for the NE reuptake. Unlike NE, ^{123}I-MIBG is not metabolized, allowing imaging. The uptake-1 mechanism is one of the main NE disposal systems, and its malfunction may lead to abnormal catecholamine concentration in the synaptic cleft[77]. Cardiac scintigraphy and positron emission tomography are the only imaging techniques with sufficient sensitivity to assess processes at picomolar concentrations in the human heart[78].

A complete cardiac ^{123}I-MIBG scintigraphy imaging protocol typically includes anterior planar scintigraphic images, obtained 15 to 30 minutes (early acquisition) and 3 to 4 hours (late acquisition), after intravenous injection of 111 to 370 MBq (3 to 10 mCi) ^{123}I-MIBG and SPECT images. Myocardial uptake and distribution is first visually assessed. After setting regions of interest (ROI) over the heart (H) and background mediastinum (M), for obtaining the mean count in each ROI, ^{123}I-MIBG uptake is semi quantified by calculating an early and late heart to mediastinum ratio (HMR). This approach provides a highly reproducible index of cardiac sympathetic activity[78]. The ^{123}I-MIBG myocardial washout rate (WOR) can be derived by comparing early and late ^{123}I-MIBG activities,

providing a parameter that reflects retention of norepinephrine by sympathetic neurons[79].

Early HMR reflects the integrity of presynaptic nerve terminals and uptake-1 function and late HMR combines information on neuronal function from uptake to release through the storage vesicles at the nerve terminals[80].

Normal values of these indices have been calculated performing ^{123}I-MIBG scintigraphy in control patients and are different between various institutions, depending on the acquisition conditions [81,82]. It was reported the value of 2.2 ± 0.3 as a normal value for H/M ratio and 1.6 (2 standard deviations below the mean) was considered as a threshold ratio below which the risk of adverse events would increase[8]. ^{123}I-MIBG WOR may reflect the turnover of catecholamines, attributable to the sympathetic drive, and measures the ability of myocardium to retain ^{123}I-MIBG. A normal value has been reported to be 10% ± 9%, with sicker patients having higher values[83]. Increased sympathetic activity in HF is associated with high myocardial ^{123}I-MIBG WOR and low myocardial early and delayed HMR[75,77,83]. Since semiquantitative analysis of cardiac ^{123}I-MIBG uptake is characterized by a low interindividual and a within-subject variability[82], semiquantitative indices of ^{123}I-MIBG scintigraphy have become valuable tools to provide information regarding the potential and actual benefit of therapeutic interventions in patients with HF[84].

In HF patients, with ischemic and nonischemic cardiomyopathy, cardiac ^{123}I-MIBG activity is a very powerful predictor of survival. A meta-analysis performed on 18 studies, with a total of 1,755 patients, provided further confirmation that patients with HF and decreased late HMR or increased myocardial WOR have a worse prognosis, when compared to those with normal semi-quantitative myocardial ^{123}I-MIGB parameters[85].

ADMIRE-HF[86], an important multicenter study, aimed to look at the prognostic value of ^{123}I-MIBG scintigraphy in HF patients, in class II-III (NYHA), with LV ejection fraction moderately to severely depressed, demonstrated that those who had late HMR $\geq$ 1.6 had a better prognosis, comparing to those with < 1.6. Preserved neuronal uptake of ^{123}I-MIBG identified a very low-risk HF population, with late H/M ≥1.60 (21% of trial subjects) associated with <1%/year incidence of cardiac death. In contrast, among the 10% of subjects with H/M <1.20, annual rate of cardiac mortality (9.6%) was 10-fold

greater. The predictive negative value of late HMR ≥1.6 was 98.2% for cardiac death at 2 years. Two-year event rate was more than twice higher in patients with H/M <1.60 (37%) compared to those with H/M ≥ 1.60 (15%).

It is still crucial to better understand the positive and negative predictive accuracy of ^{123}I-MIBG scintigraphy. The assessment of cardiac sympathetic neuronal activity by this technique can improve the knowledge of the mechanisms responsible for increased sympathetic activity in CHF, and how sympathetic overactivity exerts its deleterious action.

Other techniques of ANS Evaluation

24-hour Holter monitoring

A good tool for autonomic nervous system evaluation is 24 hours-Holter monitoring, using the analysis in time and frequency domains of heart rate variability (HRV), which consists in the beat-to-beat variations in the R-R interval on the ECG. The HRV represents the autonomic balance between the sympathetic and parasympathetic pathways action on the intrinsic rhythm of the sinoatrial node of the heart[87.88].

It is known that variability reduction is a negative prognostic parameter, associated with increased mortality in patients with myocardial infarction and systolic heart failure[89.90]. Decreased HRV results from a relative decrease in parasympathetic activity in relation to sympathetic activity, and this facilitates arrhythmogenesis[91].

A variety of methods have been devised to quantify HRV, ranging from simple statistical descriptions to complex nonlinear mathematical algorithms.

Spectral analysis of HRV is a widely used method to assess ANS function. In a continuous electrocardiographic (ECG) record, each QRS complex is detected, and the so-called normal-to-normal (NN) intervals (meaning all intervals between adjacent QRS complexes resulting from sinus node depolarizations), or the instantaneous heart rate is determined. Simple time–domain variables calculated include the *mean NN interval*, the mean heart rate, the difference between the longest and shortest NN interval, the difference between night and day heart rate, and more. The simplest variable to calculate is the *standard deviation of the NN interval* (SDNN), meaning the square root

of variance. Since variance is mathematically equal to the total power of spectral analysis, SDNN reflects all the cyclic components responsible for variability in the period of recording. In many studies, SDNN is calculated over a 24-h period and thus encompasses both short-term high frequency variations, as well as the lowest frequency components seen in a 24-h period[87]. Other commonly used index is SDANN (SD of the average of RR intervals over each 5 minutes period), RMSSD (root mean square of the differences in the adjacent RR intervals differences) and pNN50 (% of adjacent RR intervals that are >50 msec apart)[87,88].

Exercise Testing

During exercise, heart rate increases, mediated by sympathetic activation and, after exercise suspension, heart rate recovery is mediated by vagal reactivation. The rate at which HR declines appears to reflect the sympathetic drive recovery, which was necessary during exercise[92]. Increased vagal activity associated with a faster HR recovery, has been shown to be associated with a decrease in risk of death[93,94]. For this reason, several recent studies have looked at HR recovery after exercise as a prognostic tool. Consideration has been given to the role of HR in recovery as a predictor of mortality. Cole et al.[95] looked at 2,428 adults who had been referred for exercise cardiac scintigraphy over six years. They found that using a drop of 12 beats/min at 1 min after exercise as the definition of an abnormal response, a relative risk for death of 4.0 was observed. The group with a value ≤12 had a mortality of 19%, while the group with a HR decrease >12 had a mortality of 5% over a six-year period. The study employed the symptom-limited Bruce protocol with a 2-min cool-down walk. Patients on betablockers were included in the study and no difference was seen in the ability of the test to discriminate between low and high-risk patients in those patients on therapy. The authors used all-cause mortality and performed survival analysis with and without censoring of interventions (coronary artery bypass grafting and percutaneous transluminal coronary angioplasty) and found no difference in results.

These investigators also studied a different population of asymptomatic patients, enrolled in the Lipid Research Clinics Prevalence study, who underwent exercise testing using Bruce protocol. Heart rate recovery, measured at 2 minutes after exercise, continued to be a strong predictor of all-cause mortality: patients with a value ≤ 42 bpm had a mortality rate of 10%, while patients with >42 had a mortality rate of 4%, at 12 years of follow-up[96]. Given the differences in methods, direct comparisons between the two studies were not possible, but this second study confirmed HR recovery as a powerful prognostic measurement.

To further elucidate the power of HR recovery in distinct populations, the group of Nishime and Cole published another study using patients referred for standard exercise treadmill testing. Using the same methods as the original study, they found similar results. Patients with an abnormal HR recovery <12 bpm at 1st minute after exercise had 8% mortality at 5.2 years[97].

Shetler *et al*.[98] in order to validate the best cut-off for HRR, found, in 2993 male patients, with or without prior MI, no prior bypass surgery, referred for clinical exercise testing, without cool-down walk but instead with prompt supine placement, that a HR drop inferior to 22 beats/minute at 2 minutes recovery identified a high-risk group of patients. They considered this parameter validated at 1 or 2 minutes of recovery, as a prognostic treadmill measurement, and therefore advised that it should be recorded as part of all treadmill tests. They also considered that the prognostic power of this measurement does not appear to be affected by beta-blockade, but its cut-point is most likely affected by the selected population and protocol. A score including HR recovery, METs, age and history of typical angina pectoris was superior to cardiac catheterization data for predicting prognosis.

Interestingly, Arena *et al*.[99] demonstrated, in HF patients that, HRR_1 appeared to outperform peak VO_2, LVEF, and HF etiology in predicting risk of death or hospitalization, adding significantly to the prognostic value of the VE/VCO_2 slope. They confirmed that HRR may therefore provide important additive information to traditional exercise test variables during the clinical examination of patients diagnosed with HF.

Evidence from these studies suggest that the rate at which parasympathetic tone increases after the cessation of exercise appears to heavily influence the time course of HRR and undoubtly to prognosis.

Renin–angiotensin–aldosterone system

Hiperactivity of the renin-angiotensin-aldosterone system (RAAS) occurs in patients with heart failure. Alterations in blood volume, arterial pressure, and cardiac and vascular structure and function can be expected. Adaptive mechanisms may be helpful, in the short term, in maintaining suddenly decreased cardiac function but, in the long term, chronic stimulation of the RAAS leads to adverse cardiovascular effects and progression of heart failure. The actions of angiotensin II (Ang II) include vasoconstriction, cardiac remodeling, fibrosis, endothelin generation and sympathetic activation[100]. For therapeutic purposes, inhibition of the RAAS can be performed at many levels.

As cardiac function becomes less efficient, decreased renal perfusion and sympathetic stimulation result in increased secretion of renin by the juxtaglomerular apparatus in the kidney. Renin is a circulating aspartic proteinase that cleaves a peptide bond in angiotensinogen, converting it into the decapeptide angiotensin I (Ang I). Renin is a highly specific catalyst for this first, rate-limiting step of the RAAS. *Danser et al.* have demonstrated elevated renin activity and prorenin levels in hearts from patients with dilated cardiomyopathy (DCM)[101]. Cardiac level of angiotensinogen, inversely correlated with renin concentration, was one third of the level of control hearts. Cardiac renin and angiotensinogen levels were high and cardiac tissue and plasma levels of renin were closely correlated, suggesting that renin was taken up by the circulation. In a dog model, it was shown that RAAS was upregulated in heart failure. Ventricular pacing led to increased levels of renin, angiotensinogen and angiotensin-converting enzyme (ACE), with increased p53 binding to angiotensinogen and AT1-receptors[102].

Immunofluorescence studies have identified a renin receptor that binds to the mesangium of the kidney and to the subendothelium of the coronary arteries in man. The binding of renin to its receptor induced an increase in the efficiency of conversion of angiotensinogen to Ang I, providing a mechanism for intracellular and interstitial Ang II formation[103].

Some insight into the importance of renin in cardiovascular disease can be obtained from experience with renin inhibitors[104,105],.

The role of renin inhibition in prevention of cardiovascular mortality and morbidity is not yet known, but the possibility that it may provide more complete blockade of tissue RAS than ACE inhibition opens up intriguing possibilities[105].

A major question regarding the role of angiotensin II (Ang II) in the pathophysiology of heart failure has been whether other enzymes, in addition to angiotensin-converting enzyme (ACE), could contribute to the local production of Ang II in the heart. Specifically, there was a controversy as to whether the major Ang II-forming enzyme within the heart is ACE or chymase, a chymotrypsin-like serine protease that is synthesized and stored in the cardiac mast cells and is not affected by ACE inhibitors. According to a paper from Kokkonen[106], the role of chymase Ang II is generated, not only by ACE, but also by chymase, a chymotrypsin-like serine protease which catalyses the conversion of Ang I to Ang II, 20 times faster than ACE, and not affected by ACE inhibitors[100]. While *in vitro* experiments have suggested that chymase is the main source of Ang II, *in vivo* experiments more than 70% of Ang II formation was shown to be inhibited by an ACE-I. For chymase to exert its action in the heart, the cardiac mast cells, inside which chymase is stored in secretory granules) must be stimulated to degranulate[106].

Cardiac mast cells density and mediator release were compared in heart tissue from patients with idiopathic dilated and ischaemic cardiomyopathy and in normal control subjects without cardiovascular disease, by Patella *et al*[107]. They found that mast cells density was much higher in the hearts of cardiomyopathy patients, and that their histamine and tryptase content was higher than in control hearts. There is mounting evidence to link mast cells and chymase with extracellular matrix remodeling in heart failure.

Several studies have been performed with AngII inhibitors. Sukenaga and colleagues found that administration of the chymase inhibitor NIC3201 caused potent inhibition of the inflammatory response, tissue Ang II formation and fibrosis[108]. Matsumoto *et al.* used the highly specific chymase inhibitor SUNC8257 to test the role of chymase in a dog model of tachycardia-induced heart failure[109]. Dogs treated with the chymase inhibitor during pacing had significantly less collagen deposition, their left ventricular diastolic function was better and their cardiac mast cells density was decreased compared to the control group. The chymase inhibitor reduced Ang II levels by 18% and

collagen volume fraction (representing the level of fibrosis) by 60%, suggesting that chymase-mediated tissue fibrosis cannot be due solely to its Ang II-forming potential. These inhibitors may help to define the role of mast cells and mast cells chymase in heart failure. The presence of functionally active chymase could explain the observation that plasma Ang II levels can return to normal during long-term treatment with ACEIs. This idea is supported by Petrie's finding of an increased inhibition of conversion of Ang I to Ang II in resistance arteries from patients with heart failure with the combination of chymostatin and ACE-I compared with ACE-I alone[110].

Aldosterone is synthesized from cholesterol, predominantly in the adrenal cortex. Cytochrome P-450 enzyme (2 forms) catalyse the final step of these synthetic pathways[111]. P-450 11ß-hydroxylase (11ß-OHase) synthezises corticosterone from 11-deoxycorticosterone (DOC) in the *zona fasciculata* and *reticularis* and is mainly regulated by adrenocorticotropic hormone (ACTH). P-450 aldosterone (Aldo)-synthase, which catalyses synthesis of aldosterone from DOC, is present only in the *zona glomerulosa*. Its activity is mainly controlled by Ang II and potassium and more weakly by ACTH and sodium[112,113]). While prolonged administration of ACTH causes a decrease in aldosterone synthesis, it is also a potent stimulator of its synthesis in some acute conditions[114].

Aldosterone used to be thought of exclusively as a hormone that acted on the kidneys to retain sodium, excrete potassium, and increase systemic blood pressure. However, since the 1990s, there has been a revolution in our understanding of the physiology and biology of aldosterone. The first novel finding was the discovery of, extra-adrenal sites of aldosterone synthesis[115], including the brain, vascular tissue, and the myocardium[116,117]. In heart failure, myocardial tissue synthesizes even more aldosterone. The second novel finding was that mineralocorticoid receptors (MR) activated by aldosterone are widespread in the body including the brain, vascular tissue, and the myocardium. This means that aldosterone may act in a paracrine fashion in many tissues, meaning locally synthesised aldosterone may act on local aldosterone receptors to mediate local (mostly adverse) effects. These range from vascular endothelial dysfunction to inflammation widespread tissue injury and repair[118].

From the evidence in animal studies, it has long been recognized that high blood pressure can damage tissues in the brain, kidneys, and heart, but we know now that Ang

II and aldosterone can also damage tissues, independent of their effects on blood pressure. In other words, Ang II and aldosterone produce tissue damage in two ways: directly and also by increasing blood pressure. Rocha *et al.*[119] have shown that selective aldosterone blockade, at doses of aldosterone blockers that do not alter the blood pressure, or adrenalectomy markedly reduce tissue damage in saline drinking, spontaneously hypertensive rats. The most striking data in this regard are from a recent study in which eplerenone (a selective aldosterone blocker), at a dose that does not affect the systolic blood pressure, reduced brain injury in these animals and prolonged their survival. This tissue damage is not unique to the brain because it has also been shown in renal tissue and, more recently, in the heart.

Aldosterone mechanisms related to HF are synthesized, as following:

- ANS imbalance – decreased parasympathetic and increased sympathetic activity
- Endothelial dysfunction – interference with nitric oxide (NO) production
- Vascular effects – direct action on muscle layer and adventicia (besides endothelium), increased angiotensine vascular response
- Coronary dysfunction – altered responsiveness of bradychinin and acethylcoline
- Myocardial Fibrosis – stimulated myocardial fibrosis

A harmful effect of aldosterone is the reduction of parasympathetic activity. The evidence for this is threefold. Wang *et al.*[120] showed conclusively, in animal studies, that aldosterone directly reduces baroreceptor discharge from the carotid sinus and reduces the heart rate response to changes in blood pressure. Yee and Struthers[121] found confirmatory evidence in humans in that aldosterone halved the reflex bradycardic response to pressor stimuli. Macfadyen *et al.*[122] found, in their studies of HF patients, that spironolactone reduced heart rate and increased heart rate variability, which is a strong evidence for aldosterone having parasympatholytic effects.

A hypothesis regarding sympathovagal imbalance is that vascular NO might be a key regulator of autonomic balance. The observation that Nω-monomethyl-L-arginine (L-NMMA) produces baroreceptor dysfunction in humans is in favor of this concept. It could turn out that the autonomic effects of aldosterone may also be attributable to

aldosterone's vascular effects and, in particular, its ability to reduce vascular NO. Not only aldosterone decreases parasympathetic activity, but also increases cardiac sympathetic activity. This is particularly relevant because the parasympathetic nervous system is believed to oppose the arrhythmogenic effects of the sympathetic nervous system[123]. Indeed, the UK heart trial has shown clearly that autonomic dysfunction, as measured by heart rate variability, is a strong independent predictor of mortality in heart failure[124].

Aldosterone is also related to endothelial dysfunction. Ikeda *et al.*[125] showed that aldosterone interferes with nitric oxide production in animals. Farquharson and Struthers[126] showed that spironolactone improves endothelial dysfunction in patients with HF by increasing endogenous vascular NO.

Rajagopalan *et al.*[127], in animal models of atherosclerosis, presented a similar result with the specific aldosterone blocker, eplerenone. Eplerenone was demonstrated to decrease nicotinamida adenina dinucleótido hidreto/nicotinamide adenine dinucleotide phosphate-oxidase (NADH/NADPH oxidase) dependent free radical production and has potential anti-atherosclerotic effects. The concept of the anti-atherosclerotic effect of aldosterone blockade is further strengthened by the recent studies in apolipoprotein E-deficient (E(0)) mice. Aldosterone administration to E(0) mice was shown to increase macrophage oxidative stress and atherosclerotic lesion development, while blocking of the MR and inhibition of tissue ACE and/or the angiotensin receptor-1 reduced aldosterone deleterious pro-oxidative and pro atherogenic effects[128].

Most results have been obtained after chronic aldosterone blockade by spironolactone or eplerenone. In patients with heart failure, spironolactone improved acetylcholine-mediated endothelium-dependent vasodilatation and increased NO bioactivity[125]. Bauersachs *et al.*[129] demonstrated that the addition of spironolactone to ACE inhibition in rats with heart failure resulted in improvement in endothelial vasomotor dysfunction that can be attributed to the normalization of NO-mediated relaxation through the beneficial modulation of NO balance and superoxide anion formation. One of the main mechanisms, which is thought to account for aldosterone blockade producing its benefits in Randomized Aldactone Evaluation Study (RALES)[130] and Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS)[131], is that it improves endothelial/vascular function[132]. This was shown, not only in moderate

HF patients, but also in mild HF patients on all standard therapies (ACEIs, betablockers, statins, aspirin)[133]. A number of animal and human studies have suggested that changes in potassium concentrations may directly improve endothelial function by NO pathway, as the study of Tadei *et al*. [134]. The beneficial effect of aldosterone blockade was also shown by Farquharson and Struthers[135] to be independent of the effect of ENaC function and the associated increase in plasma potassium concentrations. These authors went on to show that endothelial-derived vasorelaxation was also blunted when normal volunteers were acutely infused with aldosterone at a dose that does not alter blood pressure[136]. Therefore, evidence supports the idea that there is an interaction between aldosterone and NO, such that aldosterone reduces NO bioactivity while aldosterone blockade increases NO bioactivity. This suggests that aldosterone induced tissue fibrosis may be a repair process after aldosterone has produced tissue injury due to endothelial dysfunction possibly causing tissue micro infarcts. Regarding vascular effects, there are suggestions that aldosterone may act not only on endothelium of blood vessels, but also on the smooth muscle layer, and perhaps even on the perivascular adventitial layer[137]. It now appears that aldosterone can increase vascular angiotensin responses, enhancing the binding to Ang II, to its receptors, amplifying the Ang II response[138].

Another prime adverse effect of aldosterone is its ability to stimulate fibrosis in the myocardium. Brilla *et al.* [139] showed that aldosterone induces biventricular fibrosis in rats and that myocardial fibrosis could be prevented by spironolactone at a dose too low to change the blood pressure.

Studying myocardial fibrosis in humans is difficult, but investigators have recently proposed that plasma levels of pro-collagen type III amino-terminal peptide (PIIINP) may be an useful index of myocardial collagen turnover [140]..

Zannad *et al.*[141] found that, in the RALES trial, spironolactone had its main effect in those patients who had high initial levels of, which were reduced by spironolactone. This evidences that beneficial effects on cardiac death for aldosterone blockade are mediated, at least to some extent, by myocardial collagen reduction. Therefore, it is likely that aldosterone causes patchy myocardial fibrosis, which could lower the threshold for malignant ventricular arrhythmias in HF. This was the major reason for spironolactone reducing mortality in the RALES trial. It is not known how much of this fibrosis is a direct effect of aldosterone and how much is a result of aldosterone-induced

vasculopathy causing tissue ischemia and injury. However, reducing left ventricular fibrosis is not the only beneficial effect of aldosterone blockade on left ventricular abnormalities. It appears that aldosterone blockade also reduces left ventricular hypertrophy and improves left ventricular dilatation/dysfunction[142]. Importantly, these beneficial effects occur in HF and in hypertension. Therefore, a large part of the beneficial effect of aldosterone blockade could be the result of its effects on left ventricular hypertrophy, left ventricular dysfunction, and left ventricular fibrosis.

Bradykinin (BK) has also plays a role. ACE catalyzes the degradation of bradykinin to inactive metabolite. Thus ACEIs block BK inactivation, so the concept that BK may have an important role in cardiovascular function and disease gained credence with the observation that the beneficial effects of ACE inhibition are obtained despite normal Ang II levels. BK inhibition has been indicated to attenuate the hypotensive effect of ACE inhibitors[143-145].

The activated RAAS contributes to left ventricular remodeling, alteration in ventricular mass, size and shape resulting from myocardial injury or overload. At the cellular level, myocyte hypertrophy, fibroblast hyperplasia and increased collagen deposition may be observed[146]. On the contrary, the inhibition of the RAAS mitigates left ventricular remodeling in the failing heart[147,148]. AT1-receptor blockade may have additional effects on limiting left ventricular remodeling when co-administered with an ACE-I.

Ang II induces the expression of genes for collagen and fibronectin, integrins and focal adhesion kinase phosphorylation, and increases of plasminogen activator inhibitor (PAI-1) and TGF-β expression in fibroblasts[149]. These effects appear to be mediated through the AT1-receptors. In terms of AT2-receptors, recent findings demonstrate that these mediate cell growth inhibition[150] cell differentiation[151] and tissue regeneration[152,153]. The extracellular matrix is partially maintained by the matrix metalloproteinases (MMPs) that degrade collagen by tissue inhibitors of matrix metalloproteinases (TIMPs) that inhibit them and plasminogen activator inhibitor (PAI-1) that prevents MMP activation[154].

Ang II has been shown to increase PAI-1 and TGF-β production from cardiac fibroblasts[155] and to increase TIMP-1 production from endothelial cells[156]. Thus the RAAS regulates homoeostasis of the extracellular matrix. Ang II elicits complex and highly regulated cascades of intracellular transduction in the heart.

A great deal of work has beeing carried out into the genetics and molecular biology of the various components of RAAS and this may offer in the near future new therapeutic possibilities.

Inflammation

Citokynes

Increased circulating and intracardiac levels of pro-inflammatory cytokines have been associated with chronic heart failure[157-160]. Coronary artery disease (CAD) and non ischemic dilated cardiomyopathy (DCM), the most frequent HF etiologies, are believed to have an inflammatory pathogenic basis[161].

Cytokines are low molecular weight proteins, which function as mediators of immune and inflammatory reactions. They are involved in recruiting cells to inflammatory sites and stimulating cell division, proliferation and differentiation[162]. Following an initial insult, the increased production of pro-inflammatory cytokines, including TNF-α, IL-6, IL-1, and IL-18, jeopardizes the surrounding tissue through propagation of the inflammatory response and direct effects on the cardiac myocyte structure and function. Citokines increased levels show a positive correlation with disease severity[157-160]. In response to injurious insults, cardiac structural cells contribute further to production of pro-inflammatory cytokines[160]. Cardiac myocyte hypertrophy, contractile dysfunction, cardiac myocyte apoptosis, and extracellular matrix remodeling contribute enormously to the development and progression of chronic heart failure[163-165].

To introduce novel therapeutic strategies that modulate the inflammatory response in the context of the failing heart, it is of prime importance to determine the contributions of TNF-α, IL-6, IL-1, and IL-18 in mediating cardiac adaptive and maladaptive responses, as well as delineating their downstream intracellular signaling pathways and their potential therapeutic implications[161].

Tumor Necrotic Factor - TNF-α

Tumor Necrotic Factor, TNF-α (157-amino acid cytokine), is produced by a variety of immune and non immune cells in response to inflammatory and infectious stimuli[161].

Cardiac cells are capable of producing it, when submitted to adequate mechanical, ischemic or infectious stimuli[166-170].

TNF-α has both physiological and pathological effects. In physiological concentration, it regulates local defense mechanisms and provokes regional tissue homeostasis[171-173]. It exerts subsequently protective effects, as an autocrine or paracrine mediator[174]. At higher concentrations, acts in an endocrine manner, resulting in cachexia[175], contributing to multiple organ failure[176], intravascular coagulation and thrombosis[177] and septic shock[178].

TNF-α acts biologically through two membrane receptors: tumor necrosis factor receptor type 1 (TNFR1), which is expressed dominantly and mediates the majority of cytotoxic and deleterious effects, and type 2 (TNFR2), which mediates the cytoprotective effects of the heart[179]. The cytoprotective effects of TNF-α might be due to induction of manganous superoxide dismutase (MnSOD), which neutralizes and detoxifies the cytotoxic oxygen free radicals[180,181].

Maladaptative responses to TNF-α are cardiac myocyte hypertrophy, contractile dysfunction, cardiac myocyte apoptosis and extracellular matrix remodeling[161].

TNF-α can influence the expression of IL-1 and IL-6[182], which then stimulates the hypertrophic growth response[183,184].

InterleuKin – IL-6

Interleucin-6, IL-6 cytokines, including IL-6, IL-1, LIF and others, are pleiotropic cytokines with redundant properties. All members of the IL-6 superfamily share gp130 dimerization that triggers several downstream signaling cascades. These cytokines are expressed in a wide variety of tissues and organs, mediating proliferation, growth, differentiation, survival and apoptosis signals[185-188].

Activation of gp130 exerts cytoprotective effects and improves survival via inhibition of apoptotic signaling pathways[187-188]. Current evidence points out the importance of 3 major signaling cascades as the mediator and regulator of gp130-induced cytoprotective effects, although their exact contributions are not clear[161].

IL-6 related cytokines, with subsequent activation of gp130 signaling, contribute to cardiac myocyte hypertrophic growth response[187,188]. IL-6 is also a potent mediator of

myocardial depression, which in turn potentiates the cardiodepressant effects of TNF-α and IL-1[189].

Il-6 and leukemia inhibitor factor (LIF) significantly reduce collagen synthesis and total collagen content in adult cardiac fibroblasts, respectively[190].

Role of Inflammation in HF

Enhanced expression and release of inflammatory cytokines such as TNFα, interleukin (IL)-1, IL-6, IL-18, cardiotrophin-1, adhesion molecules and Fas- ligand, as well as several chemokines in the failling heart[191-196]. have shown that there is an important role for inflammation in the pathophysiology of systolic HF.

Plasma levels of inflammatory cytokines and chemokines appear to be elevated in direct proportion to deterioration of functional class (NYHA classification) and to cardiac performance (left ventricular ejection fraction)[192,197].

Several of these mediators have been found to give prognostic information beyond that of traditional risk markers[198].

A series of experimental studies have revealed that the biological effects of cytokines may explain several aspects of the syndrome of chronic HF. The pathogenic role of inflammatory cytokines in chronic HF is supported by various transgenic mouse models. Notably, systemic administration of TNF-α in concentrations comparable to those found in the circulation of HF patients has been shown to induce a dilated cardiomyopathy-like phenotype in animal models[199]. Cardiac-specific overexpression of TNF-α has been found to promote a phenotype mimicking several features of clinical HF such as cardiac hypertrophy, ventricular dilation and fibrosis, as well as several biochemical and cellular dysfunctions[200].

In myocardial contractility depression, TNF-α plays a central role[201-204]. It induces apoptosis in cardiac myocytes, which contributes to the progressive LV wall thinning and adverse cardiac remodeling[199,200,205]. At the molecular level, sustained overexpression of TNF-α activates both intrinsic and extrinsic apoptotic pathways and leads to progressive loss of anti-apoptotic proteins[206].

Alterations in the collagen quantity and quality have been crucial in cardiac remodeling and progressive LV dysfunction[207,208]. Importantly,TNF-α decreases collagen synthesis

and pro-collagen mRNA in rats, *in vitro*, and causes imbalance between extracellular matrix (ECM) synthesis and degradation through dysregulation of the degradative enzymes, matrix metalloproteinases (MMPs) and the multifunctional endogenous tissue inhibitors of MMPs (TIMPs), major determinant of pathological ECM remodeling[209].

Studies in gene-modified mice have also shown a link between IL-6 and its receptor subunit glycoprotein (gp) 130, which is common to several cytokines in the IL-6 family and the development of HF[185].

Inflammatory cytokines may modulate myocardial functions by a variety of mechanisms including stimulation of hypertrophy and fibrosis through direct effects on cardiomyocytes and fibroblasts, impairment of myocardial contractile function through direct effects on intracellular calcium transport and signal transduction through β-adrenergic receptors, induction of apoptosis and stimulation of genes involved in myocardial remodeling. Inflammatory mediators may also contribute more indirectly to the progression of HF through impairment of bone marrow function with secondary anemia, inappropriate endothelial cell activation and impairment of peripheral muscle with secondary induction of systemic inflammation and reflex abnormalities in HF[157].

While the excess of these mediators is maladaptive, too little may also be harmful, illustrating the challenges for imunomodulating therapy in HF.

Endothelial Function

Endothelial vasodilator function is a surrogate for endothelial health[210].

Endothelial dysfunction is commonly defined as the inability of the artery to sufficiently dilate in response to an appropriate endothelial stimulus. It is considered an early predictor of cardiovascular disease[211-213] and might be the causal pathological mechanism of various metabolic diseases, referred to as “the common soil hypothesis”[214]. Endothelial dysfunction has been shown to be impaired in type II diabetes mellitus, hypertension, hypercholesterolemia, obesity, renal failure and coronary artery disease[215-219], being these conditions associated with considerable

morbidity and mortality. As a consequence, it is expected to gain interest as a potential target for intervention.

Endothelial function mechanisms

The knowledge on endothelial function has been slowly evolved over recent decades Previously, endothelial dysfunction was thought to be limited to impaired endothelial NO production and bioavailability in response to physiologic stimuli, thereby resulting in impaired vasodilatation. In addition to this initial idea of primary impaired NO signaling pathways, it is now known that the diagnosis of endothelial dysfunction also takes into account dysfunction of many other autocrine and paracrine signaling pathways leading to miscommunication between endothelial cells (EC) and cardiomyocyte (CM)[220]. Stressors, such as diabetes[221], hyperlipidemia/atherosclerosis[222], hemodynamic stress (shear stress)[223], inflammatory cytokines[224], and ischemia/coronary artery disease[225], can alter endothelial function and thereby actively affect EC-CM communication and ultimately lead to cardiac failure[226]. Therapeutic intervention to prevent the adverse outcomes of endothelial dysfunction and EC-CM miscommunication, ultimately preventing HF, is subject of intense clinical investigation.

Cardiac endothelial cells (EC) rely on diverse routes of communication. Endocardial EC and capillary EC share an active blood-heart barrier and influence neighboring cardiomyocyte (CM) through juxtacrine and paracrine signaling, whereas coronary vascular EC act indirectly on CM through changes in coronary vasomotor tone and consequent alteration of blood flow[227]. Interestingly, either cell can initiate communication; CM can act as secretory cells and are the source of many paracrine signals that affect EC. Among these are endothelin-1 (ET1), fibroblast growth factors, adenosine, and heme oxygenases—which regulate vascular tone—thus coordinating myocardial metabolic requirements[228]. Additionally, CM paracrine signaling, namely vascular endothelial growth factors, affects growth and development of coronary vessels. Myocardial ischemia and heart failure (HF) require vascular growth to match the increased energy demands[229], and failure of vascular adaption leads to progressive cardiac dysfunction [228]. Likewise, EC play pivotal roles in the bidirectional interactions between these two major cell types.

EC act as sensors for shear stress to regulate vascular tone. Cardiac EC can regulate contractile properties of CM. Several autocrine and paracrine signaling molecules are responsible for this important physiologic mechanism. They produce NO, endothelin-1 and neuroregulin-1[220].

Nitric oxide, produced from L-arginine by three different NO synthase isoenzymes, is a pivotal signaling molecule between EC and CM. Under physiologic conditions, neuronal (nNOS) and endothelial (eNOS) NO synthase produce the majority of NO. During inflammation, inducible NO-synthase (iNOS) significantly augments NO production[230]. Interestingly, oxygen free radicals produced during ischemia-reperfusion limit NO bioavailability without significantly affecting NOS activity[231]. Similar to its effects on smooth muscle, NO affects the onset of ventricular relaxation, allowing for optimization of ventricular pump function[232]. Although CM express both nNOS and eNOS, the vast majority of NO production comes from the EC, exceeding that of CM by greater than 4:1[233].

The role of NO in healthy myocardium, as well as the adaptive changes during pathology, have been widely published[234]. Furthermore, studies in mice have provided substantial evidence that eNOS derived NO attenuates ischemia-reperfusion injury[235,236], and ultimately improves survival during HF[237].

NO bioavailability is also necessary for a vast majority of cardioprotective effects and interventions. Ischemic preconditioning[238] perfectly exemplifies such an NO-dependent cardioprotective intervention[234]. Interestingly, several drugs used for the treatment of hypercholesterolemia[239,240] or even erectile dysfunction[241] improve NO bioavailability and are cardioprotective.

NO, generated in the endothelial cells, is one of the most important factors regulating vascular function[242]. There are 3 isoforms of NO, generated by NO-synthase: neuronal NOS, inducible NOS and endothelial NOS (eNO). This last one is the most important in endothelial cells to regulate vascular tone. It is responsible for vasodilation, which results in the lowering of peripheral resistance and increase of perfusion. eNOS expression was significantly reduced in animal models of HF[243,244]. Its activity is upregulated by an increase in flow-mediated shear stress associated with physical exercise due to a complex pattern of intracellular regulation: acetylation[245], phosphorylation[246], translocation to the caveolae[247].

After animal[248] and culture studies[249], exercise or shear-stress was demonstrated to upregulate eNOS activity in humans[250].

The glycocalyx on the luminar side of endothelial cells seem to play an important role in signal transduction of increased shear stress and eNOS activation[251,252]. In addition, vascular endothelial growth factor receptor 2 leads to eNOS phosphorilation and higher eNOS production[253]. HDL is another factor known to modulate eNOS via phosphorylation[254]. This HDL-induced activation is impaired in patients with HF[255], but also in CAD[256], diabetes[257] and an ET program of 12 weeks is able to restore the HDL-mediated eNOS activation[255].

The bioavailability of NO not only depends on generation but also is influenced by ROS (reactive oxygen species)-mediated breakdown[258]. eNOS also generates itself ROS in the vascular system[259]. NOS uncoupling has been implicated in heart failure[260], arteriosclerosis[261] and diabetes [262].

Endothelial dysfunction and the failing heart

Coronary and peripheral endothelial dysfunction are present in both, ischemic and non-ischemic, HF[263-265]. Independently of the initial underlying HF etiopathology, EC dysfunction plays a major role in the progression of the disease and has important prognostic value on clinical outcomes[266-268].

During HF, endothelial dysfunction is present, not only in coronary EC, but also in the arteries of skeletal muscles, explaining the early fatigue and exercise intolerance in HF[269]. EC-mediated vasoconstriction contributes to the increased peripheral vascular resistance in chronic HF[270]. In addition, dysfunctional endothelium has been observed in renal, mesenteric, and pulmonary vasculature, which is consistent with the notion that global EC dysfunction plays an important role in HF[271] .

Both, preclinical and human studies, emphasize the importance of coronary endothelial dysfunction during HF. In particular, the identification of impaired vasodilatory responses supported the notion that decreased NO impairs myocardial perfusion and indirectly contributes to the progression of HF[263,272]. Yet, cardiac endothelial dysfunction, similar to coronary vascular endothelial dysfunction, is an early event in the progression to fulminant HF[273]. Indeed, high concentrations of neurohormones cause selective

damage to cardiac EC, and depress mechanical performance of the adjacent myocardium. Moreover, secretion of traditional paracrine/autocrine factors is indispensable for EC-CM communication, and, such secretion is altered during acute, progressing, and stable HF[274,275]. Recent evidence has shown that the activation of the β1-adrenergic- protein kinase A pathway and the ET-1-protein kinase C pathway is crucial in positively modulating full developed force-frequency response (FFR) in cardiac muscle (276), and dysregulation of FFR is a hallmark of HF[277]. Thus, our silo-style view of vascular vs. cardiomyocyte dysfunction requires reevaluation.

Clinical Assessment of Endothelial Function and impact of interventions

Endothelial function plays a key role in vascular health and endothelial dysfunction is an early event in atherogenesis, making endothelial function testing, a mean for cardiovascular risk stratification, a valuable tool for clinicians[278]. Presently, there is no test to evaluate directly the impact of EC-CM interactions on cardiovascular health. The goal of developing a non-invasive and effective test for endothelial function has proven to be challenging[279]. Several investigational methods are mentioned in the next paragraphs.

The impact of exercise on endothelial function has been studied[280]. Arterial-level shear stress (>15 dyne/cm^2) at the outer edges of vessel bifurcations can stimulate the vasculature to produce factors ultimately promoting an atheroprotective gene expression profile[281]. Non-invasive techniques to further assess the impact of exercise on endothelial function are being intensively studied, including magnetic resonance imaging[282].

As another potential non-invasive measurement of endothelial function[283], some have used positron emission tomography scanning to identify increased vascular inflammation. Chronic inflammation is a well-known risk factor for cardiovascular disease[284,285]. Many groups investigated the potential impact of anti-inflammatory drugs (like non steroid anti-inflammatory drugs, NSAIDs) on endothelial function. A salicylate reduces vascular inflammation, and increases brachial artery flow-mediated dilatation in overweight/obese patients in a NFκ B-dependent manner[286]. Concerns, however, have

been raised about NSAIDs risks[287]. Further studies need to evaluate the safety of anti-inflammatory therapy on the cardiovascular system.

Endothelial dysfunction, commonly described as the inability of the artery to sufficiently dilate in response to an appropriate endothelial stimulus, can presently be assessed by the measurement of flow-mediated dilation (FMD) of the brachial artery after occlusion of the blood flow or by measurement of the arterial pulse wave at a finger artery.

High resolution ultrasonographic imaging of the brachial artery assesses endothelium-dependent flow-mediated vasodilation. Although the exact mechanisms causing FMD are not entirely known, the main mechanism inducing FMD is thought to be an increase in shear stress, leading to the release of nitric oxide from endothelial cells which causes blood vessel dilation[288]. This technique allows estimation of various interventions effectiveness[289]. A recent study used this method to test the relative effectiveness of two different endothelial-directed drugs and found that the technique was, indeed, effective[290]. It has been widely used and shown to be a suitable tool to assess endothelial dysfunction. However, the method has several disadvantages: it is operator dependent[291], and as FMD is measured at one arm only, there are no possibilities to correct for potential measurement-induced changes in the systemic hemodynamics, such as those resulting from alterations in the autonomous nervous system tone.

To overcome these problems, the EndoPAT (*Itamar, Israel*) was developed. This device allows non-invasive measurement of vasoreactivity without the disadvantages of conventional ultrasound measurement. The EndoPAT detects plethysmographic pressure changes in the finger tips, caused by the arterial pulse, and translates these to a peripheral arterial tone (PAT). Endothelium-mediated changes in vascular tone after occlusion of the brachial artery are reflecting a downstream hyperemic response, which is a measure for arterial endothelial function[292]. Measurements on the contralateral arm are used to control for concurrent nonendothelium-dependent changes in vascular tone. In addition, the EndoPAT provides also a measure for arterial stiffness: the augmentation index (AI). The rationale for AI is the following: as a pressure wave moves through the arterial tree, it encounters impedance resulting in a reflected wave that moves back toward the heart and may augment peak systolic pressure[293]. Arterial stiffness increases pulse wave velocity, causing early reflection of this waveform[294]. The EndoPAT-derived

augmentation index (AI) provides a measure of arterial stiffness by considering the timing and magnitude of this wave reflection in the digital pulse[295].

Calculated from baseline resting pulse waves, AI represents the relative contribution of augmented pressure due to wave reflection to the pressure wave form. The software automatically identifies inflection points distinguishing the systolic peak (P1) and the reflected peak (P2) for the calculation of this ratio and converts it into a percentage (P1–P2/P1*100)[294]. Because AI is inversely related to heart rate[296], values are sometimes mathematically adjusted to represent arterial stiffness at a standard heart rate of 75 beats per minute (AI@75). PAT arterial stiffness measures are associated with abnormal ventricular-vascular coupling[294] and correlate well with AI measures from other devices[297]. In theory, the EndoPAT appears to be a useful device in clinical research, as the test is easy to perform, not operator-dependent, and with comprehensive automatic analysis. In a group of 89 adult patients suffering from chest pain, peripheral arterial tone correlated positively with FMD[292]. The evaluation of cross-sectional relations of digital vascular function to cardiovascular risk factors, in the Framingham Heart Study, showed a significant inverse relation between endothelial function, as determined by the EndoPAT ("EndoScore" or reactive hyperemia index, RHI), and multiple cardiovascular risk factors (male sex, body mass index, total/HDL cholesterol, diabetes, smoking, and lipid-lowering treatment)[298]. The EndoScore was reported to be significantly decreased in patients with coronary artery disease, hypertension, hyperlipidemia, diabetes, glucose intolerance, and tobacco users (group sizes of 15 to 70 subjects)[292,299-303]. Several EndoPAT studies have demonstrated an improvement in endothelial function as a result of lifestyle modification (smoking cessation, and dietary change)[304-307] or prolonged pharmacological intervention[308-309].

Generally, augmentation index, calculated from carotid, aortic, or radial artery pressure waves using conventional techniques, is a reliable and a reproducible measure to define arterial stiffness[310]. However, the influence of variables such as heart rate and vasomotor tone of the arterial system can affect the variability of the technique[311]. When using the EndoPat, the intraindividual variability in AI is substancial (coefficient of variation of 37%), which limits its usefulness to assess interventions[312]. Compared to arterial stiffness, RHI proved to be a more stable measure over time (coefficient of variation 13%). Interestingly McCrea *et al.*[313] showed for the first time that PAT

measures of endothelial function are highly repeatable across intervals greater than 1 week, in healthy adults.

Left ventricular remodeling

Cardiac remodeling is considered to be implicated in the pathophysiology of HF progression[314-318]. The term *ventricular remodeling* refers to alteration in ventricular architecture, with gradual increases in end-diastolic and end-systolic left ventricular volumes, wall thinning, and chamber geometry change to a more spherical, less elongated shape, usually associated with continuous LV ejection fraction decline. In clinical practice, changes in ejection fraction, LV end-diastolic and end-systolic volumes, LV mass, and sphericity index are usually used as surrogate parameters for remodeling or reverse remodeling evaluation.

This process is driven on a histologic level by a combination of pathologic myocyte hypertrophy, myocyte apoptosis, myofibroblast proliferation, and interstitial fibrosis[319-321].

The concept of cardiac remodeling was initially developed to describe changes which occur in the days and months following myocardial infarction, extending to nonischemic cardiomyopathies, such as idiopathic dilated cardiomyopathy, suggesting common mechanisms for the progression of cardiac dysfunction[322-323].

The process of cardiac remodeling is influenced by hemodynamic load, neurohumoral activation, and other factors, still under investigation. The myocyte is the major cardiac cell involved in the remodeling process. Other components include the interstitium, fibroblasts, collagen, and coronary vasculature; relevant processes also include ischemia, cell necrosis and apoptosis[318] Due to continuous maladaptive remodeling, myocardial dysfunction with increasing LV volumes is usually a progressive condition, where even mild initial dysfunction may develop to severe heart failure over a time course of months to years, independently of the initial cause[324-326]. Functional polymorphisms in modifier genes relevant for disease progression may impact on the remodeling process. The results of cardiac remodeling include progressive worsening of systolic and diastolic function, development of mitral regurgitation, and increased propensity for arrhythmias. A hallmark in remodeling is alteration in the phenotype of

the myocytes with reexpression of a fetal gene program, defective excitation–contraction coupling, and disturbed intracellular Ca^{2+} handling. Despite myocyte hypertrophy, this leads to defective contractile function, which may contribute to further progression of myocardial remodeling[327].

Very little information, concerning subcellular remodeling in the development of heart failure is available in the literature. Since subcellular remodeling in different experimental models of HF is dependent upon the species of animals employed, as well as the different stage and type of HF[326-328], it is difficult to implicate remodeling of any particular organelle in the genesis of cardiac dysfunction. Since some studies[329-336] have indicated progressive alterations in extracellular matrix, sarcolemma membrane, sarcoplasmic reticulum, and myofibrils at early, moderate, and late stages of HF, in both cardiomyopathic hamsters and myocardial infarction in rats, it is likely that remodeling of these subcellular organelles is involved in the progression of HF. Some investigators have suggested the role for remodeling of extracellular matrix, cytoskeletel system, and myofilaments in heart failure and dilated cardiomyopathy[337-339], whereas others have shown remodeling of sarcoplasmic reticulum, at early stage, and of myofibrils, at late stage of heart failure[340]. Likewise, Ca^{2+}-handling abnormalities due to remodeling of sarcoplasmic reticulum and sarcolemma membrane, as well as changes in extracellular matrix and responses of myofibrils to Ca^{2+}, have been observed in both systolic and diastolic forms of human heart failure[341-343]. Remodeling of one or more subcellular organelles may explain the transition of compensatory cardiac hypertrophy to heart failure[344-345]. Ding *et al*.[346] have reported that the transition from cardiac hypertrophy to heart failure due to volume overload is associated with altered intracellular Ca^{2+} homeostasis as a consequence of sarcoplasmic reticulum remodeling.

Prevention of remodeling has been documented in coronary artery disease. It is much less clear whether remodeling may be reversed, once it has developed.

In the case of myocardial infarction, the most effective strategy to prevent pathological remodeling is immediate reperfusion during acute phase to minimize myocardial damage. The strategy of late reperfusion is controversial[347], although some studies showed reduction in infarct expansion and pathological remodeling, both in animal models[348] and in humans[349]. Subsequent early initiated pharmacological therapy with

ACE inhibitors[328] and beta-blockers[350] may prevent or slow ventricular remodeling after myocardial infarction.

In fact, as mentioned, in view of the lack of sufficient information, it is difficult to confirm the involvement of any particular subcellular organelle in ventricular remodeling for LV systolic or diastolic dysfunction. This knowledge could lead to specifically directed therapies for inhibiting remodeling progression. Preventing or reversing maladaptive remodeling, implicated in HF progression, is an accepted therapeutic target, reason why it is so important to continue investigating subcellular remodeling.

4.2. Heart Failure - pharmacologic and non pharmacologic therapy

The evolution of therapy for heart failure has become increasingly complex.

It began by pharmacological therapy, with various developments, although more recently, non pharmacological treatment, like internal cardiac defibrillator and resynchronization therapy, mitral valve intervention, cardiac transplant, left ventricular assistance and exercise therapy have been introduced[351]

Despite pharmacologic efficacious regimens and mechanical interventions, HF remains among the leading causes of mortality in the world.

Pharmacologic Therapy

The 2012 European Society of Cardiology (ESC) guidelines[26], 2013 American College of Cardiology/American Heart Association (ACC/AHA) updated guidelines[352] and the 2010 Heart Failure Society of American (HFSA) guidelines[353] with varying levels of evidence, recommend the following:

- Diuretics (to reduce edema by reduction of blood volume and venous pressures) and salt restriction (to reduce fluid retention) in patients with current or previous heart failure symptoms and reduced left ventricular ejection fraction (LVEF) for symptomatic relief

- Angiotensin-converting enzyme inhibitors (ACEIs) for neurohormonal modification, vasodilatation, improvement in LVEF, and survival benefit
- Angiotensin receptor blockers (ARBs) for neurohormonal modification, vasodilatation, improvement in LVEF, and survival benefit
- Hydralazine and nitrates to improve symptoms, ventricular function, exercise capacity, and survival in patients who cannot tolerate an ACEI/ARB or as an add-on therapy to ACEI/ARB and beta-blockers in the black population for survival benefit
- Beta-adrenergic blockers for neurohormonal modification, improvement in symptoms and LVEF, survival benefit, arrhythmia prevention, and control of ventricular rate
- Aldosterone antagonists, as an adjunct to other drugs for additive diuresis, heart failure symptom control, improved heart rate variability, decreased ventricular arrhythmias, reduction in cardiac workload, improved LVEF, and increase in survival
- Digoxin, which can lead to a small increase in cardiac output, improvement in heart failure symptoms, and decreased rate of heart failure hospitalizations
- Anticoagulants to decrease the risk of thromboembolism
- Inotropic agents to restore organ perfusion and reduce congestion
- Ivabradine is indicated in stable, symptomatic chronic heart failure patients with LVEF of 35% or lower, in sinus rhythm with resting heart rate of 70 bpm or higher, and either on maximally tolerated doses of beta-blockers or with contraindication to beta-blocker use, leading to 18% drop in the risk for cardiovascular death or hospitalization for worsening heart failure

Antiarrhythmic agents and calcium channel blockers may be used, as necessary, carefully. These drugs can have cardiodepressant effects and may promote arrhythmia, with only amiodarone and dofetilide shown as not adversely affect survival.

Calcium channel blockers can worsen heart failure and may increase the risk of cardiovascular events; only the vasoselective calcium channel blockers have been shown not to adversely affect survival.

Reverse remodeling in heart failure with medical therapy

Both ACE inhibitors and beta-blockers have been shown to slow, or even temporarily reverse, remodeling in heart failure[333]. Initial results with ACE inhibitors clearly indicated benefit, but were controversial with respect to true reverse remodeling: Captopril did not lead to a reduction in LV volume, but attenuated progressive LV dilatation, in 59 patients after anterior myocardial infarction and ejection fraction inferior to 45%. In contrast, compared to placebo, captopril produced a significant reduction in LV end-systolic volume and an increase in ejection fraction after 3 months of therapy in 100 patients after myocardial infarction[328]. Substudies of the SOLVD trial evaluated the effect of enalapril versus placebo on serial changes in left ventricular volumes and ejection fraction. Despite the small numbers included, these studies demonstrated early and sustained reduction in left ventricular volumes after initiation of ACE inhibition in both asymptomatic and symptomatic patients[326,329]. In the larger echo substudy of the Studies of Left Ventricular Dysfunction (SOLVD) trial, enalapril treatment prevented further LV enlargement, associated with a slight reduction in LV mass, over a follow-up period of 12 months[330]. However, in the Survival And Ventricular Enlargement (SAVE) trial, captopril attenuated LV dilatation within the first year after myocardial infarction, but not in the second year of follow-up[354]. These results indicate that patients may escape from the beneficial effects of ACE inhibition on maladaptive remodeling after prolonged periods of time. Taken together, ACE inhibitors attenuate or prevent further remodeling, and may induce modest reverse remodeling in subgroups of HF patients. Whether more complete inhibition of the RAAS system by combining ACE inhibitors with AT1 antagonists and aldosterone receptor blockers is more effective for induction of reverse remodeling awaits clarification. The large Valsartan Heart Failure (ValHeFT) trial[332] demonstrated that the combination therapy of AT1 receptor antagonist Valsartan with ACE inhibitor was more effective than ACE inhibitor therapy alone to induce reverse remodeling, in patients with symptomatic systolic heart failure. Both, ejection fraction increased and left ventricular end-diastolic dimension decreased significantly, more with combined RAAS blockade, during prolonged (>24 months) periods of time. Compared to ACE inhibitors, even more pronounced effects on reverse remodeling may be observed with beta-blockers, in heart failure patients[333]. Hall *et al.*[334] reported a progressive increase in ejection fraction, regression of dilatation and hypertrophy, and restoration of a more elliptical chamber shape, in patients treated

with metoprolol over a time course of 3–18 months. Reverse remodeling with β1-adrenoceptor blockade was confirmed in a substudy of the Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF trial), where patients treated with metoprolol CR/XL were followed by magnetic resonance imaging. Significant decreases in LV volumes, and increases in ejection fraction were observed after 6 months, compared to the placebo group[355].

Similarly, using the non-selective adrenoceptor blocker carvedilol, Olsen *et al.*[336] reported reverse remodeling associated with improved symptoms in chronic heart failure.

In the echo substudy of the Australia–New Zealand trial in patients with ischemic cardiomyopathy and severely depressed LV function[337], carvedilol treatment over 12 months was associated with smaller LV end-diastolic and end-systolic volumes and LV increased ejection fraction. A meta-analysis of all available beta-blocker trials showed an average of 29% relative increase in ejection fraction, irrespective of the etiology of heart failure[338].

In these studies, with selective or non-selective beta-blockers, reverse remodeling was observed, even in the presence of baseline therapy with an ACE inhibitor. These data support the hypothesis that the prognostic benefit of beta-blocker therapy in heart failure is related to the potential to prevent and reverse left ventricular remodeling.

Reverse remodeling with beta-blocker therapy has also been documented on the subcellular level in isolated human myocardium. Some beta-blockers may increase the number of β-adrenergic receptors, which are downregulated in heart failure[339].

Lowes *et al.*[340] demonstrated an association between normalization of myocardial gene expression for SERCA2a and α- and β-myosin heavy chains and improvement in ejection fraction and clinical status in patients with idiopathic dilated cardiomyopathy under beta-blocker therapy. Reiken *et al*.[341] showed that beta-blocker therapy partially restored diastolic filling, β-adrenergic responsiveness, and ryanodine release channel function in patients with dilated or ischemic cardiomyopathy. These data provide insight into subcellular mechanisms for reverse remodeling with beta-blockers.

Novel pharmacological approaches for reverse remodeling in heart failure have been developed, like pharmacological blockade of the sarcolemmal sodium/hydrogen exchanger, which may prevent or reverse maladaptive remodeling[342]. Cariporide

prevented fibrosis and heart failure in β1-adrenergic transgenic mice[343] and reversed isoproterenol-induced hypertrophy in rats[344]. Another sarcolemmal sodium/hydrogen exchanger isoform 1 (NHE1) inhibitor, EMD-87580, induced reverse remodeling in a rat model of post-myocardial infarction heart failure[345].

Taken together, ACE inhibitors and even more pronounced, beta-blockers, induce reverse remodeling in heart failure, and this may in part explain improved clinical outcome. In the future, more complete blockade of neuroendocrine activation, e.g. by addition of AT1-antagonists and aldosterone receptor blockers, possibly in combination with complementary pharmacological approaches, may be even more effective for reverse remodeling[327].

Non pharmacologic Therapy: Cardiac Resynchronization

CRT history and trials

Electric stimulation of the heart goes back for more than two and a half centuries[356]. Ten years after Zoll[357], who used closed chest external stimulation to treat patients with cardiac arrest for complete atrioventricular block, transvenous permanent pacing was developed[358]. Despite the success, it became clear that both dual-chamber pacing and single-chamber pacing often conduced to impairment of cardiac function, which required correction. Optimization of the time interval between atrial and ventricular stimulation in dual-chamber pacing improved ventricular performance. After that, attention was directed to the synchronicity of ventricular contraction. It had long been appreciated that patients with HF and intraventricular conduction defects, particularly left bundle–branch block, were poorly responsive to the usual HF therapy and that their prognosis was especially poor[54].

Some years later, aware that univentricular pacing impaired intraventricular conduction and caused QRS prolongation and dyssynchrony of ventricular contraction, Bakker *et al*.[359], reported the beneficial hemodynamic and clinical effects of biventricular pacing in 5 patients with severe HF and left bundle–branch block.

Later, in 1998, Daubert *et al.* [360], describing a completely transvenous CRT implantation, with over-the-wire technique from Auricchio[361], opened a new era for CRT.

In 2001, the safety and efficacy of CRT were first addressed by the Multisite Stimulation in Cardiomyopathies (MUSTIC) trial[362] and Pacing Therapies in Congestive Heart Failure (PATH-CHF)[363] study.

In the Multicenter InSync ICD Randomized Clinical Evaluation (MIRACLE –ICD), the first study which explored the addition of CRT-D to ICD, the benefit on quality of life and clinical NYHA class was demonstrated[364].

The Comparison of Medical Therapy, Pacing and Defibrillation in Heart Failure (COMPANION) trial[365], the first trial to compare CRT-P and CRT-D with OPT, demonstrated a 20% reduction in death or hospitalization from any cause. Total mortality was inferior with CRT-D. In an outcome study regarding the patients of COMPANION[366], mortality in NYHA class IV patients, however, was not reduced, by either CRT-P or CRT-D.

Two large randomized controlled trials (COMPANION)[365] and Cardiac Resynchronization in Heart Failure Study (CARE-HF) [4], involving patients with severe HF, showed that CRT resulted in the reduction of symptoms and HF hospital admissions and in increased survival. These trials established CRT as a treatment for HF NYHA III-IV, impaired LV function and enlarged QRS. Device treated patients had similar characteristics in both studies, however in COMPANION the control group patients were on optimized pharmacologic therapy (OPT) and ICD and in the CARE-HF wer only on OPT.

While CRT is now widely used for the treatment of patients with advanced HF, LV dysfunction and QRS >150 ms, clinical investigators have been seeking to extend the indications.

Efficacy of CRT-D in mild HF was suggested by CONTAK CD study, which demonstrated LV reverse remodeling in classes II-IV, NYHA[367].

In MIRACLE ICD II study [368], which included patients of class II, NYHA, CRT-D induced LV reverse remodeling compared with ICD.

In Multicenter Automatic Defibrillator Implantation Trial (MADIT) [369], randomizing patients in class I and II to CRT-D or ICD , demonstrated that CRT-D reduced combined event total mortality or HF events by 34% (no difference in total mortality) .

In the Resynchronization Reverses Remodeling in Systolic Left Ventricular Dysfunction (REVERSE) study [370], patients in class I-II with primary prevention ICD indications were randomized to CRT-on/off. CRT-on improved LVEF and reduced HF hospitalizations.

The Resynchronization/ Defibrillation for Ambulatory Heart Failure Trial (RAFT)[371], similarly to REVERSE, compared CRT-D with ICD in class II-III patients. Total mortality or HF hospitalization occurred in 33.2% in CRT-D vs 40.3% in ICD group (hazard ratio:0.75;95% confidence interval:0.64-0.87).

Due to the low mortality, MADIT-CRT and REVERSE studies did not demonstrate independent effect on mortality in class I and II patients[369,370]. Also, the 5-year follow-up of REVERSE study showed low mortality in patients randomized to CRT-on[372]. These data, as that from the RAFT study[371], provided evidence regarding CRT in the early stages of HF.

In the registry Improve the Use of Evidence-based Heart Failure Therapies in the Outpatient Setting (IMPROVE HF)[373], that device therapy was shown to add incremental survival value to OPT in advanced HF.

In CARE-HF study[4], for patients in the non-CRT group, annual mortality rate was 12.6%, worse than in many cancers.

Despite some authors[369-371]have demonstrated that CRT can retard disease progression in mild HF, we know that CRT uptake is low in this population[374].

Despite the cost of the device, the cost-effectiveness of CRT-P and CRT-D, based on the evaluation of several studies[375] proved identical to many medical interventions.

Presently, CRT is an accepted treatment for patients with moderate-to-severe HF and intra-ventricular conduction delay, identified by a QRS interval of 120 msec or more on a 12-lead electrocardiogram (ECG). This prolonged QRS interval occurs in up to a third of the patients with severe systolic HF and is associated with dyssynchronous LV contraction, leading to impaired emptying and, in some patients, to mitral regurgitation. Abnormal atrioventricular coupling (prolonged PR interval) and interventricular dissynchrony, identified by echocardiography, may also occur. CRT with atrial-synchronized biventricular pacing often improves cardiac performance immediately, by increasing stroke volume (SV) and reducing mitral regurgitation.

This intervention has led to a significant reduction in mortality and morbidity, in selected patients, as reviewed in multiple trials[375]. On the basis of these trials, current European guidelines[376] recommend CRT for patients with severe symptoms (NYHA class III or IV) despite optimized pharmacologic therapy, ejection fraction (EF) persistently 35% or less, sinus rhythm, and QRS duration of 120 msec or more (class I

recommendation). More recently, it has expanded to other HF groups, despite not being recommended as a first class indication. Questions remain, regarding the possible benefits of CRT in patients with milder degrees of HF and QRS prolongation, as well as in HF without QRS prolongation, eventhough with echocardiographic dyssynchrony[377].

CRT mechanisms

Patients with HF often have an associated delay in electrical conduction that leads to discoordinate contraction of the heart. Whether identified by QRS duration or echocardiography, dyssynchrony is an independent predictor of increased mortality in patients with HF[378,379].

Remodeling involves both global cardiac dysfunction and regional stress disparities within the left ventricle, with relative unloading of the early activated (typically septal) region and high load in the late (lateral) contracting regions[380]. Late systolic stretch of the early stimulated wall reduces net ejection and can worsen functional mitral regurgitation[381].

While it is intuitive that a synchronized ventricular contraction should be superior to a dyssynchronized one, it remains to be determined whether or not there is also a molecular basis for the observed improvement in ventricular function with CRT. Kirk and Kass[382] reviewed systematically the evidence for and against specific cellular and molecular changes that occur with ventricular dyssynchrony and how these changes respond to CRT. Biventricular pacing has been shown to cause reverse ventricular remodeling, with reduction in biomarkers, including NT-proBNP and markers of extracellular matrix metabolism[383].

Findings from animal models of LV dyssynchrony[384] revealed regional variations in myocyte hypertrophy, blood flow, and oxygen consumption. Cardiac resynchronization therapy, during which biventricular stimulation is applied, was developed to counter this pathophysiology. Resynchronization therapy induces reverse remodeling on the cellular and organ level.

Patients with heart failure and asynchronous wall motion due to intraventricular conduction delay are at increased risk for exacerbated pump failure. Biventricular and left ventricular cardiac resynchronization therapy can re-coordinate contraction,

thereby acutely improving systolic ventricular function and energetic efficiency. CRT acutely improves net systolic function without affecting diastolic volume[377].

Yu *et al.*[385] provided the first evidence that CRT induces reverse remodeling when they showed that LV end-systolic and end-diastolic volumes decreased already after 1 month, and continued over a period of several months of CRT. These authors also demonstrated that this adaptation persisted some time, even if pacing was temporarily suspended. Transitory suspension of pacing for 3 months, led to acute decrease in systolic contractile function, but without enlargement of ventricular volumes. However, if pacing was maintained off for one more month, chamber dilatation and remodeling restarted.

Results of subsequent randomized trials, already presented, demonstrated that these changes were associated with improved clinical outcome. CRT has been shown to improve symptom class, exercise capacity, and quality of life even in patients already receiving optimal pharmacological therapy. Albeit the mechanisms of benefit remain poorly understood, reverse remodeling of the failing ventricles may be considered a major factor.

In another small trial using radionuclide scintigraphy, Toussaint *et al*. [386] reported an immediate, albeit not significant effect of resynchronization on ejection fraction (from 17.8±6.3 to 19.9±8.3), which further increased (to 24.2±10.8%;$p<0.05$) after 1 year of CRT. These data indicate that CRT has both immediate and long-term effects on ventricular function, the latter possibly associated with reverse remodeling.

Larger, chronic and blinded/controlled studies have confirmed the reverse remodeling effects of CRT on chamber geometry[387]. Recently, reverse remodeling with CRT was also observed in the MIRACLE study[7]. In this trial, the effect of CRT on chamber geometry and remodeling was assessed by Doppler echocardiography in 323 HF patients (mean EF, 24±7%) on optimized medical heart failure therapy including ACE inhibitors (>90%) and beta-blockers (>55%). 172 patients were randomized to CRT-on and 151 patients to CRT-off. CRT induced significant and progressive reverse remodeling over 6 months: left ventricular end-diastolic and end-systolic volumes as well as mitral regurgitation decreased, associated with an increase in ejection fraction. In addition, LV mass decreased and myocardial performance and left ventricular sphericity index improved. Reverse remodeling was associated with improved NYHA functional class, exercise

capacity, and quality of life. It occurred regardless of the cause of heart failure, but more extensively in patients with a nonischemic origin. This observation was confirmed in the echocardiographic substudy of the MUltisite STimulation In Cardiomyopathies (MUSTIC) trial[388].

The mechanisms for reverse remodeling with CRT may comprise: reduced wall stress due to reduced inhomogeneities in regional contractile activation[382], decreased sympathetic nerve activity[389] and increased metabolic efficiency[390].

Penicka *et al*.[391] identified pulse-wave tissue Doppler derived intraventricular and interventricular asynchrony as the best predictive factors for reverse remodeling of the left ventricle during CRT. Nevertheless, reverse remodeling was less likely to occur in the presence of rather dilated chambers: Stellbrink *et al.*[392] identified CRT non-responders with respect to reverse remodeling to have significantly higher baseline LVED volume, as compared to responders (351±52 vs 234±74ml). This is consistent with the observation that patients with large ventricles may be non-responders to medical therapy regarding reverse remodeling[393].

The real importance of evaluating reverse remodeling in HF patients as an endpoint after therapy relates to the fact that death rate in recent HF trials, with optimal pharmacologic therapy, is low when there is reverse remodeling. Regression of maladaptive remodeling might serve as a surrogate marker for morbidity and mortality and success of therapy. This hypothesis is supported by the analysis and review of several studies[327,394]. Nevertheless, most of the major heart failure trials to date have only correlated treatment and cardiac function and have not directly demonstrated a causal relationship between improvement in cardiac function and improvement in long-term health outcomes.

Autonomic nervous system function significance, as a mechanism for CRT benefits, was addressed. Several studies have shown that cardiac resynchronization therapy (CRT) improves sympathetic function in patients with HF accompanied by reduced systolic function. CRT improves β-adrenergic function and up regulates presynaptic receptor function. Also, biventricular pacing was shown to reduce muscle sympathetic nerve activity when compared with right ventricular pacing[390] or right atrial pacing[395]. These beneficial effects persisted at 6 months after resynchronization therapy[396]. Cha *et al.*[397] examined the effect of CRT on neuro-hormonal integrity by studying cardiac presynaptic

sympathetic function, as determined by nuclear cardiac imaging modalities (^{123}I-MIBG scintigraphy), in patients with HF who received CRT and found that CRT reverses cardiac autonomic remodeling by up-regulating presynaptic receptor function, as evidenced by increased ^{123}I-MIBG heart/mediastinum ratio and attenuated heart/mediastinum washout rate, with concomitantly improved HRV[397]. Najem *et al.*[398] found that sympathetic inhibition induced by chronic CRT was acutely reversed when patients were shifted from a synchronous to a nonsynchronous mode. This was observed only in patients who responded to CRT, even more than an year after initiation of the therapy. The mechanism by which CRT inhibits sympathetic activity is intriguing because correction of the electric and mechanical dyssynchrony with biventricular pacing does not directly block the sympathetic nervous system. It is probable that biventricular pacing improves cardiac function over time and thus reduces sympathetic drive[37].

Besides the effect on ANS, CRT has been shown to reverse the apoptosis caused by dyssynchrony[399] and to enhance a variety of mitochondrial enzymes associated with the augmentation of ATP production[400]. Also, it has been demonstrated that CRT significantly improves endothelial function through the improvement of cardiac output in HF patients, compared to optimal medical therapy[401].

While new therapeutic strategies, such as miniaturized impeller pumps for chronic unloading, gene therapy, or stem cell therapy, in reverse remodeling awaits characterization, resynchronization therapy undoubtly induces reverse remodeling on the cellular and organ level in selected patients.

CRT Response Definition

The definition of CRT response varies widely between studies, with numerous criteria to define a positive response in the literature.

"Echocardiographic response" is typically assessed by quantifying the change in left ventricular ejection fraction[402-405] or left ventricular end-systolic volume [406-411], usually 3 to 6 months after CRT implantation. We know, however, that some patients may respond later[386].

"Clinical response" is defined by the improvement in New York Heart Association functional class[403,412-414] or increase in the distance walked in 6 minutes[414], 3 to 6 months after CRT implantation.

Some studies have defined response to CRT as a combination of several clinical measures[416-418] or as a combination of both clinical and echocardiographic measures[419]. The heterogeneous approach to defining response to CRT severely limits the ability to generalize results over multiple studies and it constitutes a potential barrier to progress in the field. Fornwalt has addressed this issue by investigating the agreement among the numerous published CRT response criteria[420], which are the following:

Echocardiographic

- Var. LVEF >5 units
- Var. LVEF>15% (relative)
- Var. LVESV>10%, no HF, no death
- Var. LVESV>15%
- LVESV >115% baseline
- Var. LVEDV>15%
- Stroke volume>15%

Clinical

- NYHA >1
- NYHA >1, no HF, no death
- NYHA >1 and 6MWT>25%
- NYHA >1 and 6MWT>25%, no HF death
- 6MWT>10%, no HF death, no transplant
- Two of the following:NYHA >1, 6MWD>50 m, QOL>15

Clinical composite score

- Combined LVEF>5 units or 6MWT>50 m and NYHA>1 or QOL>10

The poor agreement found among these response criteria severely decreases the ability to generalize results from multiple studies. The authors applied different criteria to the

426 patients enrolled in the Predictors of Response to Cardiac Resynchronization Therapy (PROSPECT) study[411]. The major findings of this analysis were: the 26 most-cited publications on predicting response to CRT used 17 different primary response criteria, and the level of agreement, independent to chance, among 15 of these response criteria was poor, in 75% of the times, and strong in only 4%, in the PROSPECT study patients; agreement between echocardiographic and clinical response criteria was poor and nearly equal to the level of agreement expected by chance; the percentage of patients defined as having a positive response to CRT ranged from 32% to 91% for the 15 response criteria; 99% of patients were classified as responders by at least 1 of the 15 criteria, whereas 94% were classified as a nonresponders by at least 1 criterion[420].

Similarly to Fornwalt *et al.*, Aaronaes *et al.* assessed response criteria to CRT[421]. Different response criteria to CRT gave response rates ranging from 33–96% and 31–94% at six and 12 months, respectively. Other previous studies had previously reported different rates of response to CRT when different definitions of response were used, within the same population. Even looking at the PROSPECT study[411], which reported 56% of patients as echocardiographic responders (reduction in LVESV of at least 15%), whereas 69% as clinical responders (improvement in the clinical composite score), which are not necessarily coincident patients, we understand poor agreement of response.

Facing this, which method should we use in the future to determine whether a patient benefited from CRT?

Because heart failure is a debilitating life-threatening disease, an effective heart failure therapy should treat both symptoms and quality/duration of life. Measures of "response" to CRT should either directly measure outcomes or have a surrogate relationship with benefits in heart failure symptoms, quality of life, and duration of life. The clinical composite score[422] is a measure of response that accounts for all of these factors and may be the best overall choice for defining response in future CRT trials.

Other inconsistencies in defining response to CRT is the length of follow-up period after which a patient is deemed either a responder or a nonresponder. Some studies focused on short-term, 1 to 2 days, response [402.405,423,438,424], whereas most focused on 3-months[406-416,419] or 6-months [403-405, 411-418, 392, 425-429] response. However, CRT has been

shown to have persistent, increasing benefits with a longer mean follow-up period of 29.5 months[430].

Another issue is about mortality: whether death should be considered a nonresponse to CRT.

There are at least 3 different methods that authors have used for death as response criteria: death due to worsening HF included in the nonresponder group[415-418,425,429]; death due to any cause included in the nonresponder group[426]; death excludes patients from analysis[392,404,406,407,409,410]. Moreover, numerous publications fail to specify how death was incorporated into response criteria despite enrolling consecutive patients and following them for a 3 to 6-months period[403,412-414,419].

Although inclusion of all-cause mortality, as a criterion for nonresponse, may not be appropriate, a patient who dies of progressive heart failure should, objectively, be classified as a nonresponder. Regardless, there is no consistent method for incorporating mortality into the definition of response to CRT, and this needs to be standardized.

In resume, as expressed before, many different methods, used in the literature to define a positive response to CRT, show poor agreement among each other.

Although it is clear that cardiac resynchronization improves prognosis[4,430,431], all major trials demonstrated that at least one third of the patients are nonresponders to CRT [432,433], depending the CRT response of the definition used in the trials.

Besides the problem of nonresponse, CRT implies a relatively high additional finantial cost initially and is an invasive procedure, not exempt of complications.

Identifying patients in higher risk of death and those most likely to benefit from currently available treatment technologies, remains a challenge.

Although CRT is well established in the therapeutic armamentarium, it still offers many opportunities for research. It is important to determine what is the best criterion to define CRT response, what is the optimal time to evaluate it and how can we best predict these responders. The defined criteria should be used in a uniform and consistent basis in order to comparative analysis being possible.

Non pharmacologic Therapy: Exercise

Exercise effects in heart failure

Several years ago, exercise was considered noxious to the heart and even contra-indicated in heart failure. Presently, it is known that individuals with cardiac disease seem to be at a greater risk for sudden cardiac arrest during vigorous exercise (such as jogging) than are healthy individuals[434]. The incidence of major cardiovascular complications during outpatient cardiac exercise programs has been estimated to be 1 in 60 000 participant-hours[435]. Activities performed with continuous ECG monitoring have the lowest rates of sudden cardiac arrest compared with those that are unmonitored or only intermittently monitored[434].

Myocardial infarction is another risk associated with participation in exercise and is more likely to occur than sudden cardiac death. Approximately 4% to 20% of myocardial infarctions occur during or soon after exertion[436-438]. The adjusted relative risk, however, has been found to be greater in people who do not regularly participate in physical activity[436-437]. From the analysis of 21 exercise training studies conducted in a total of 467 patients with chronic HF[19], the overall adverse event rate seems to be low. The most common events in such patients include postexercise hypotension, atrial and ventricular arrhythmias, and worsening HF symptoms. These findings points to the need for careful patient selection, monitoring and follow-up.

Questions have been raised about possible detrimental effects of regular exercise on LV remodeling in patients after myocardial infarction. One small, nonrandomized study has shown that patients with >18% asynergy after first anterior Q-wave infarction experienced a further increase in asynergy and a decrease in EF after 12 weeks of exercise training when compared with nonexercising controls[439]. However, 2 subsequent randomized controlled trials of moderate- to high-intensity exercise training patients after large myocardial infarction have not demonstrated adverse effects on regional wall motion, LV systolic function, or LV chamber dimensions after several months of exercise[440,441]. In the larger Exercise in Anterior Myocardial Infarction (EAMI) trial[441], exercise training in patients after first anterior Q-wave infarction did not result in any significant changes in global or regional LV size for the group as a whole. Among

patients with EF <40%, spontaneous global and regional LV dilatation was seen similarly in both the exercise and control groups but was not influenced by exercise training. In another study[440] of 25 patients with reduced LV function (mean EF 32%), serial LV measurements obtained from MRI indicated no detrimental effects from 2 months of moderate-intensity cycle exercise training on LV volume or EF. Musculoskeletal injuries are common and include direct injuries such as bruises, sprains, and strains and indirect problems such as arthritis and back pain. Low-impact exercises (walking, cycling, and swimming) cause little stress on bones and joints, whereas high-impact exercises (running and aerobic dancing) cause repeated impact on the knees, ankles, and feet. Studies of injuries during exercise indicate that intensity and biomechanical impact of the activity performed are the two most important factors in determining the frequency of injuries[434].

Presently, exercise training is undoubtedly considered safe, with specific care in stable HF, and proven to be beneficial to heart failure patients in terms of physical fitness and quality of life improvement[9,442] although a clear survival benefit has yet to be demonstrated[443]. Regarding this issue, as pointed by Smart[444], the results of previously published exercise training trials may have been affected by different factors: exercise adherence is often not ideal, leading to much smaller improvements than expected; studies may have been affected by crossover to the exercise intervention in up to one-third of sedentary controls, which was the case in HF-ACTION (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training)[443]; moderate intensity continuous exercise has been the cornerstone of exercise programming, although a small volume of recent work has shown high-intensity interval exercise training to be superior for eliciting improvements in peak VO_2 and systolic heart function[445,446].

In HF-ACTION trial, the largest randomized controlled multicentre clinical trial of exercise training in patients with HF and reduced LV function, moderate continuous exercise training provided a nonsignificant reduction in the risk of the primary end point of all-cause mortality or all-cause hospitalization, despite the safety demonstration, quality of life improvement and reduction of combined endpoint of all-cause mortality and hospitalization[443]. Even the size of these benefits was modest compared to results published in smaller studies and meta-analyses.

Guidelines have been developed on the relatively large volume of data from clinical exercise training trials of moderate-intensity continuous exercise (MICE).

MICE therapy has preferably been used in people considered to be medium to high risk for cardiovascular events. Well-known reasons for this choice of exercise are the fact that the stimulus from MICE to health benefits is considered sufficient, the tolerance of MICE by most people is seen as good, not suspected to detract from exercise adherence and the risk of MICE serious medical events considered is acceptably low, whereas the considered by many high risk of high-intensity exercise[444].

More recently, and applying the knowledge obtained in sports medicine, there have been a number of high-intensity intermittent exercise (HIIE) studies and study protocols published in the scientific literature. A reference study of HIIE for clinical populations was the work of Wisloff *et al*.[445] in heart failure patients, which produced great clinical improvements, including a 46% improvement in peak VO_2, parameter regarded as the best predictor of prognosis in these patients. These authors demonstrated a superior cardiovascular effect of HIIT compared to MICE in HF patients. Wisloff *et al.* [445]study was conducted in a small sample size of 3 groups of 9 patients, and therefore, some clinicians remain unconvinced of the potential benefits of HIIE or that these programs are safe and well tolerated. The success of HIIE studies is that interval exercise allows for rest periods that make it possible for patients with heart failure to perform the total work of exercise at high intensity, being these bouts of exercise at high intensity the major determinant of adaptation. In Wisloff *et al.*[445] work, the comparison (continuous exercise) group completed exactly the same amount of work, thus removing ambiguity over dose responses.

Interval training consists of periods of high-intensity exercise alternated by periods of relative rest, which turns out possible for patients to complete short work periods at higher intensities. From a physiological point of view, high intensity interval training stimulates cardiac contractility and poses a larger impact on the endothelium and skeletal muscle mitochondrial function compared to continuous training at moderate intensity (MCT), which could add to a more favourable effect on peak VO_2[445].

Interest in clinical HIIE programs have been growing, and the SMARTEX group (Controlled Study of Myocardial Recovery After Exercise Training in Heart Failure)[447] is testing the hypothesis that a program comprising interval training at high relative

intensity would yield significantly larger effects in terms of left ventricular remodeling compared to moderate continuous exercise training.

Initial evidence indicated that HIIT results in greater improvements in left ventricular function, endothelial function and skeletal muscle, when compared to MICE, with similar adverse events during training, eventhough with rigorous screening and supervision[448]. Other groups of investigators published results on the safety of HIIT in cardiac patients, however not exclusively HF patients[449].

A meta-analysis on 7 randomized trials comparing HIIT with MICE[450] the investigators came to the conclusion that in clinically stable HF patients, HIIT is more effective than MICE in improving peak VO2, but no difference is obvious with respect to altering LV remodeling, at least regarding the improvement of rest left ventricular ejection fraction (450). This meta-analysis is only based on 180 patients in total (mean LVEF 32%), with all studies using a single-center design, so we must be cautious in the interpretation of conclusions. Larger multicenter trials comparing the different training intensities are needed, like SMARTEX. Contrarily to the expected HIIT benefits in HF patients for the just mentioned trial, the recently published multicenter trial SAINTEX-CAD[451], performed in a CAD population (most patients with no systolic LV dysfunction), demonstrated that a 12-week HIIT and MICE intervention equally improve peak VO_2, peripheral endothelial function, QoL and some cardiovascular risk factors in CAD patients. In addition, both programs seemed to be safe for CAD patients.

Resistance training, previously thought as deleterious for cardiovascular system, only recently is being considered in HF programs, besides the already used aerobic modality. For this reason not as substantial as the amount of studies regarding aerobic exercise training, resistance training (RT) has shown evidence to support the inclusion in a EXT program. HF patients have a reduction of muscle strenght and endurance, resulting from skeletal muscle abnormalities, including reduced oxidative capacity and cross-sectional area[452-454]. Improvements in muscular strenght (15-50%), endurance (18-299%), as well as peak oxygen consumption (10-18%) and 6-min walk distance (5-49%) are achievable[455-457]. RT proved to be safe and does not negatively alter LV function when prescribed at moderate intensity[454].

Several studies have been done in low to moderate risk patients, but those in high risk are the most at need, in order to live an independent life.

Mechanisms of Exercise in HF

Long-term moderate exercise training has been shown to induce reverse remodeling in patients with stable chronic heart failure, and this was associated with significant increases in work capacity and peak oxygen uptake[458]. The first large prospective randomized study to provide evidence for a training-induced reverse remodeling came from Hambrecht *et al*.[459], who demonstrated that endurance training led to reverse left ventricular (LV) remodeling, with modest improvements in LVEF from 30% to 35%, as well as reductions of LV end-diastolic diameter.

The results of these studies were confirmed in 2 meta-analyses performed in 2007[460] and 2012[461]), which showed that aerobic training, especially greater than 6 months duration, significantly reversed LV remodeling, whereas strength training alone or combined with aerobic training had no effect on reverse remodeling.

One of the first prospective studies on EXT, in HF, demonstrated that 4-6 months training did not worse LVEF and tended to improve cardiac output[11]. The extent of cardiac changes did not, however, explain completely the 23% improvement of peak VO2. Posteriorly, the systematic review of Smart and Marwick[9], confirmed the meaningful improvement of 17% (average) in the most objective reported measure of functional capacity, peak V02 ($VO_{2peak)}$. This effect was related to peripheral changes in limb perfusion related to endothelium dysfunction and oxidative muscle metabolism, neurohormonal adaptation and ventilatory function improvement, besides reverse remodeling[21,462].

Submaximal exercise capacity (SmaxExC) is also improved, as assessed by significant increase in ventilatory anaerobic threshold (VAT) and in 6-MWT distance. In HF patients (NYHA II-III), SmaxExC improvement probably is due to peripheral training adaptations in skeletal muscle mass, SMM[463]. Theoretically, by improving SMM strength, a lower percentage of maximal contraction would be used to do a similar amount of work following training, producing less blood lactate, thereby decreasing CO_2 elimination needs and thus increasing VAT. VAT improvement allows patients to exercise longer and harder without a negative effect on ventricular dynamics and could possibly improve the ischemic threshold. In severe HF patients, the true meaning of a SmaxExC

improvement, after ET program, is related to quality of life (QoL), since daily activities do not demand peak aerobic performances[464]. Tasks, such as pulling, pushing and lifting, require SMM and the strength of upper and lower limbs.

Endothelial adaptations

Endothelium- and flow-dependent vasodilatation (FMD) abnormalities are a key phenomenon in HF blunted vasodilator response. A significant improvement in endothelium-dependent relaxation has been observed in trained patients[21,22]. ExT enables the improvement of both basal endothelial nitric oxide (NO) formation and agonist-mediated endothelial-dependent vasodilatation of the skeletal muscle vasculature in HF. The correction of endothelial dysfunction (endothelium-dependent change in peripheral blood flow) is associated with a significant improvement in exercise capacity, evidenced by a 26% increase in peak oxygen uptake, as mentioned before[21].

The impaired availability of nitric oxide (NO), responsible for the impaired endothelium-dependent relaxation of peripheral resistance and conduit arteries also contribute to the reduced exercise capacity in HF and other severe symptoms[465]. Also, endothelium-independent vasodilatation abnormalities may relate to a combination of impaired smooth muscle responsiveness to NO, impaired NO diffusion to the smooth muscle and structural alterations in arterial compliance associated with HF[466-469].

It is understandable that HF patients with the most reduced endothelial function associated with the greatest sympathetic activation have a poor prognosis and need the greatest intervention.

Mechanisms of exercise training in HF, explaining reverse remodeling, have been studied. In the absence of myocardial biopsies for molecular analysis of myocardial changes induced by training, most investigators interpreted this favorable training effect as secondary to afterload reduction with reduced resting blood pressure due to improved endothelial function[21,441,458,459]. Animal models reveal, however, that there are direct myocardial effects of training that are related to signaling pathways of myocardial hypertrophy and fibrosis[437].

Neurohormonal adaptations

EXT corrects most peripheral abnormalities encountered in HF, decreasing neurohormonal stimulation without deleterious effect on LV remodeling[10].

Previous studies with HF showed that EXT reduces norepinephrine (NE) levels at rest and during exercise[12,460,464] and decreases central sympathetic nerve outflow, measured by microneurography. EXT also enhances vagal control, improving heart rate variability (HRV) and heart rate recovery (HRR), with return to better sympathetic-vagal balance (10). Compared to healthy controls, LV dysfunctional myocardium is characterized by a significant reduction of pre-synaptic NE uptake and post-synaptic beta-adrenoceptor (B-rec) density[470]. This might contribute to LV remodeling process. It is consistent with the finding that down-regulation of myocardial B-rec density, measured using positron emission tomography (PET) with 11C-CGP-12177, soon after acute MI, is predictive of LV dilatation at follow-up[471]. Myocardial B-rec density appears to be reduced in HF with dilated cardiomyopathy[472] and down-regulation of myocardial B-rec is more pronounced in patients with hypertrophic cardiomyopathy who proceed to LV dilation and HF[473]. Therefore, B-receptors down-regulation may be a general nonspecific response to stress and could be due to locally increased synaptic cleft NE. The sustained SA hyperactivity observed in HF is the consequence of several mechanisms, including increased central sympathetic outflow, modified neuronal NE reuptake and facilitated cardiovascular response to sympathetic stimulation by angiotensin II[474].

Studies in experimental HF have shown that exercise training in animals improves cardiac β-AR signaling and function, increases adrenergic and inotropic reserves of the heart and helps restoring normal SNS activity/outflow and circulating catecholamine levels[475]. Exercise training is known to increase resting vagal tone and to decrease sympathetic drive in healthy individuals. Coats *et al.*[12] showed that a similar beneficial change could be induced in HF with a 16% reduction of radiolabeled norepinephrine secretion after 8 weeks of EXT with peak oxygen uptake reduction .

Braith *et al.*and coworkers[467,477] described a 25% to 30% reduction of angiotensin II, aldosterone, arginine vasopeptide, and atrial natriuretic peptide after 4 months of walking training in patients with HF.

In a rat model of ischemic HF, the beneficial training effects on local neurohumoral balance were analyzed in the noninfarcted LV myocardium. Xu *et al*.[478] found a significant reduction of myocardial angiotensin-converting enzyme mRNA expression and angiotensin II, type 1, receptor expression after 8 weeks of treadmill EXT. This finding is of special importance given that approximately 90% of angiotensin II is produced locally in the myocardium and implies that local angiotensin II levels are significantly reduced by EXT. This reduction also translates into reduced fibrogenesis, as indicated by reduced tissue inhibitor of metalloproteinase-1 expression with un-changed matrix metalloproteinase (MMP)-1 expression and reduced collagen volume fraction in the exercised animals[478].

Inflammatory response

During HF, a derangement in inflammatory factors is evident[479]. EXT acts positively on inflammation in HF. It reduces significantly local cytokines such as TNFα and IL-6 and inducible nitric oxide synthase (iNOS) in the SMM of HF patients[480] and has a beneficial effect on peripheral inflammatory markers reflecting monocyte/macrophage-endothelial cell interaction[481]. These local anti-inflammatory effects of ET may attenuate the catabolic process associated to CHF progression. This is an important issue, since inflammatory responses play a pathogenic role in the development and progression of HF.

Nonpharmacologic Therapy: Exercise associated to CRT

Presently, little is known about EXT for advanced HF patients, with or without devices, as these patients are normally excluded from cardiac rehabilitation programs and HF studies, as happens with elderly HF patients, women and patients with comorbidities and multiple chronic conditions.

The number of HF patients implanted with an electrical device (ICD or CRT) is steadily increasing, although the beneficial exercise training effects, mechanisms and best ET protocol, for these patients, are not completely identified.

Vanhees *et al*.[482] evaluated the effect of a 3-months training programme in 92 ICD patients, compared with a control group. A total of 68% of ICD patients had a LVEF below 40%, compared with 13% of the control group ($p < 0.001$). The training programme resulted in VO2 peak 21% increase in the ICD group, with only one inappropriate ICD shock reported.

Conraads *et al.*[483] studied the effect of endurance training after CRT implantation in 17 patients (mean age 59 ± 9 years) with HF and dyssynchrony. Patients were randomized to CRT with (n = 8) or without (n = 9) exercise training. The observed increase in VO2peak was significantly greater in the trained CRT patients versus untrained (40% versus 16%; $p = 0.005$), thus demonstrating an additive effect of CRT and exercise training.

Prior preliminary studies on CRT and ExT[484,485] suggested small improvements in functional capacity, like the one performed by Patwala[23]. He studied 50 HF (NYHA class III) randomized patients who were selected to CRT, with an exercise protocol starting at 3 months after CRT and lasting 3 months. He reported, after the increased VO_{2peak} and improved skeletal muscle mass performance at 3 months after CRT, that functional capacity further improved by addition of ET to CRT device implant, as well as hemodynamic measures and QoL. Exercise showed additional improvements to CRT in these patients. No information on ANS or other potential mechanisms was provided[23].

Belardinelli *et al*.[20] evaluated the effects of a moderate aerobic exercise training program on functional capacity, quality of life and hospital readmission rate in HF patients, class II and III, with ICD and CRT. Moderate exercise looked safe and had beneficial effects after ICD and especially after CRT: improvement of peak VO2, endothelium-dependent dilation of the brachial artery and quality of life. Hospital readmission was lower in the group of exercise. Also, patients who exercised did not have any shocks, while untrained patients had 8 shocks, during the 24 months of follow-up.

The importance of the effect of anxiety on arrhythmias was demonstrated in a prospective study of van den Broeck and colleagues[486], who have shown that anxiety

predicted a 70% increase in the risk of arrhythmias in type D (or distressed) patients with an ICD. Exercise training, apart from the favourable effect on functional capacity, may also have a positive effect on anxiety in ICD patients as well, however this effect was considered still on debate in a review paper from Isaksen K *et al.* [487], including nine studies of ET in CRT/ICD patients.

The combination of EXT to CRT has not been largely investigated, yet. It is currently not completely confirmed if adding an exercise training (ExT) program following CRT provides better clinical outcomes than CRT alone.

Regarding the matter of exercise training protocol, previous experience with coronary artery disease patients[488], and data in patients with HF[489] showed that an EXT program combining aerobic exercise (AE) and resistance exercise (RE) training are more effective than an AE program alone. Also, the aerobic interval training high intensity (HIIT) showed better improvements than continuous endurance training (moderate intensity), according to Wisloff[445].

It is crucial to understand the potential additional benefit of a determined type of exercise protocol applied to these patients over the probable beneficial effect of CRT, including the effect of EXT on CRT nonresponders, which might even be more important. Moreoever, there is a need to understand the underlying mechanisms regarding the positive and negative response to this non pharmacological exercise therapy in HF.

Another important issue is the fact that most EXT studies in HF have been conducted in patients with less severe functional impairment. Very little or no information is available on NYHA class III-IV patients. It is unknown how HF with more severe functional limitations respond to EXT and, more importantly, how physiologic mechanisms can explain the improvements as a consequence of EXT. This lack of scientific information needs urgently to be overcome since this is the group of patients (NYHA class III-IV) normally targeted for CRT.

5. HYPOTHESIS AND AIMS

5.1. Hypothesis

The ain hypothesis formulated in this thesis is:

It is beneficial to associate a high intensity interval training exercise program, long duration, after cardiac resynchronization in advanced Heart Failure Patients;

Secondarly, it was hypothetized:

Several pathophysiologic mechanisms involved contribute differently to the benefit of exercise training after CRT and of CRT independently of exercise.

5.2. Aims

The primary aim of this thesis is to determine the additional effects of a long-term intervalic exercise training program on clinical functional NYHA class, quality of life, exercise functional capacity, cardiac function and reverse remodeling and major cardiac events in advanced heart failure patients after cardiac resynchronizer implant.

Secondary aim intends to evaluate the potential role and contribution of different pathophysiologic mechanisms involved in the hypothesis of exercise training intervention after CRT and of CRT by itself: endothelial function, autonomic nervous system (ANS) function, inflammatory process and apoptosis.

Primary end points were defined as:

- Clinical symptoms severity
- Quality of life
- Exercise functional capacity
- Cardiac function and remodeling
- Major cardiac events at 6 months after HIIT

Secondary endpoints were defined as:

- Autonomic nervous system function

- Endothelial function
- Inflammation and Apoptosis

6. METHODS

6.1. Study Design

This study was designed as a controlled randomized clinical trial, performed in one single centre, using a longitudinal approach with 3 moments in time for patients assessment, before CRT implant (M1), at 3 (M2) and at 6-month (M3) after the experimental therapy, long-term exercise training (EXT), which was initiated 1 month after cardiac resynchronizer implant. Consecutive stable advanced HF patients who were admitted for CRT implantation in the Cardiology Service of Hospital Santa Marta (January 2012 - March 2015) were initially screened for inclusion and exclusion criteria.
Patients who accepted to perform EXT and did not live far from the hospital were accepted for randomization.
Controlled blind randomization process to experimental exercise intervention (EXT) or not (control group, CG) was performed, based on patients stratification, according to 4 variables:

- Age : $\geq$65 or <65 years old
- Gender: male or female
- Etiology : ischemic or not
- LV systolic dysfunction severity : LVEF $\geq$ 20% or <20%

Moments of assessment in time were, as indicated:

- M1: Baseline, before cardiac implant, in the previous 4 days
- M2: at 3 months after exercise training program (4 months after CRT onset)
- M3: at 6 months after exercise training program (7 months after CRT onset)

The inclusion of a 3-months assessment moment, besides the 6-months, was based on the importance for earlier exercise data analysis necessity in order to updating the exercise intensity and also to comparing these results to those of CRT studies with

evaluation at 3 months. It turned out possible to determine if EXT/CRT effects occurred more or less early.

Exercise training program was started at 1 month after CRT device implant, if no exercise contra-indication was present, allowing patients recovery after the cardioresynchronizer intervention. Earlier exercise starting, before one month, would be too premature and unsafe for the patients, due to the technical aspects of the device implant intervention. A long, 6 months, high intensity interval training program (HIIT) was started, adapted from Wisloff[445] and according to individual prescription.

Evaluation of cardiac events occurrence at M3 was performed (7 month after cardioresynchronizer implant):

- cardiac death;
- any-cause death;
- ventricular tachycardia;
- ventricular fibrillation or cardiac arrest with hospitalization;
- HF aggravation hospitalization;
- composite event: cardiac death or cardiac hospitalization;
- composite event: cardiac death or cardiac hospitalization or ventricular arrhythmia.

Dependent variables considered were:

- NYHA class – for HF symptoms severity
- HeartQol scores, total, physical and psychologic - for quality of life, HeartQol questionnaire (Oldridge)
- LVEF, LVED Vol, LVES Vol, LA Vol, RA Vol, GLS, E/e', LV Mass, TAPSE, PSAP – for cardiac function and remodeling, determined by echocardiographic study
- CPT dur, AT time, VO2p, VE/VCO2 slope, HRmax, SBP, SBPmax, HRR1, HRR6 – for functional capacity and autonomic function, determined by Cardiopulmonary Exercise Testing
- HMRe, HMRl, WOR – for autonomic nervous function, by ^{123}IMIBG Cardiac scintigraphy

- SDNN, RSDMM, SDANN, NN50 – for autonomic nervous function, determined by 24h-Holter HRV analysis
- RHI, AI, AI@75 – for endothelial function, determined by digital tonometry (Endopat)
- BNP, hs-RCP, TNF-α, sCD40, IL-6, sFasL – for inflammation and apoptosis, determined by blood analysis
- Cardiac Events at 6 months after HIIT (at 7 months after CRT) – for short-term prognosis, determined by identification of cardiac events, considered cardiac death, total death, cardiac hospitalization (HF/ventricular arrhythmia), ventricular tachycardia, combined events (cardiac death or cardiac hospitalization and cardiac death or cardiac hospitalization or ventricular tachycardia)

In summary, the study design was characterized by:

- Longitudinal randomized controlled clinical trial
- Inclusion period: 2012 (1st January)-2015 (31st March)
- Sample size determined by statistical method (n=60 patients)
- Blind Aleatorization immediately post-CRT implant, in 2 groups: exercise training group (EXTG) and non exercise training, control group (CG)
- Exercise Training Program (HIIT), 6 months duration
- Evaluation (all exams) at M1, pre CRT implant (48 h before), at M2, 3 months after EXT onset (CPT, Echo), and at M3, 6 months after EXT onset (all exams, except Holter).

From total CRT population sample, the nonrandomized patients were also evaluated with the same approach in time and with the same methodology, despite not performing EXT intervention. These results were used for evaluation of CRT, as a prospective cohort study, besides the main randomized controlled trial.

The study was approved by the Ethics Comission of Hospital Santa Marta (attachement 1) and of University Nova, Faculty of Medical Sciences (attachement 2). The written

informed consent (attachement 3*)* was signed by all patients who participated in the study. Data protection was assured by the usual methods.

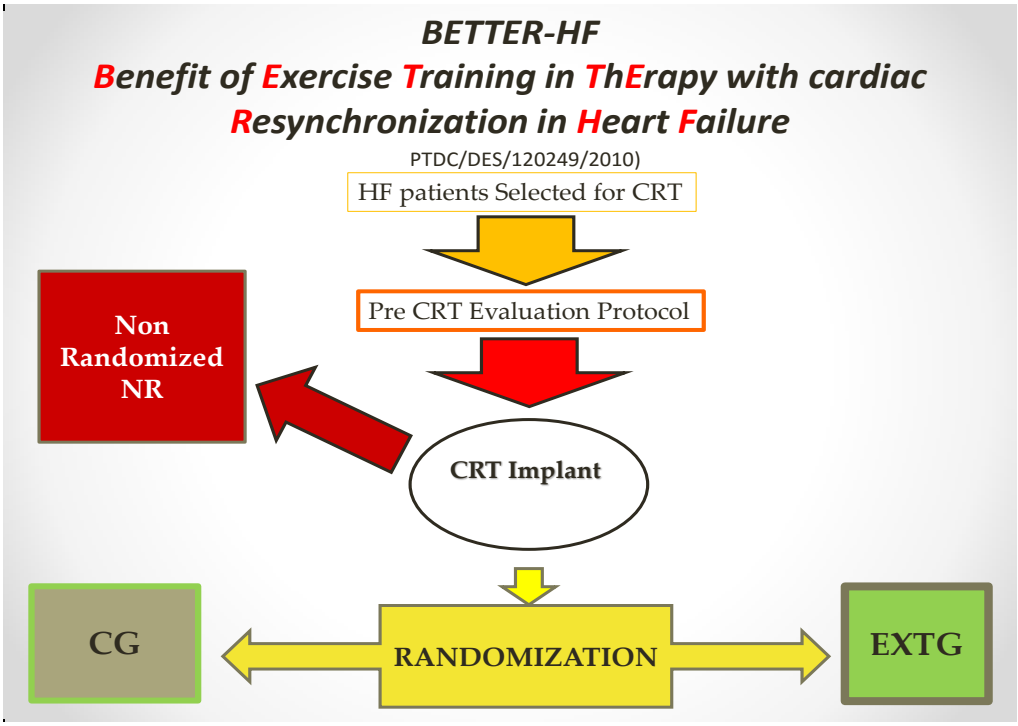

Figure 2: Study design and randomization of BETTER-HF: Patients randomized to exercise training group (EXTG), patients randomized to control group (CG), patients nonrandomized (NR)

6.2. Study Population Sample

Moderate to severe HF patients (NYHA class III-IV under optimal medical therapy), enlarged QRS (>120 msec), selected for CRT implant, were recruited from hospital Santa Marta during a period from 1st January 2012 and 31st March 2015. They all were formally invited to participate in the study after a clinical and functional screening, respecting the inclusion and exclusion criteria.

Inclusion criteria were considered:

- HF patients, class III-IV (NYHA), receiving optimal HF pharmacologic therapy
- Age > 18 years old
- LV moderate to severe dysfunction (LVEF<35%)
- QRS duration≥120 ms
- Ischemic or non-ischemic etiology
- Referred to cardiac resynchronization therapy, CRT implant at Hospital Santa Marta
- Stable condition for >1 month (no hospitalization for HF, no change in medication, no change in NYHA functional class)
- All patients must read and sign an informed consent form

Exclusion criteria were defined as:

- Geographical long distance address with difficulty/impossibility in frequent hospital displacement for ET
- Incapacitating orthopedic, neurologic or other limitations that unable the patient to exercise
- Not acceptance to participate in the study for any reason
- Inability to sign informed consent
- Previous treatment with an intravenous inotropic agent within the 30 days prior to implantation
- Unstable angina pectoris

Optimal medical therapy for HF was considered to include an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker and a beta-blocker, as guidelines recommend, unless a contraindication was evident.

Patients, who met the inclusion criteria without exclusion factors, and accepting by signing an informed consent form to participate in the study, were randomized after resyncronization therapy to either exercise program, HIIT, or control (no exercise). They were informed that this study would include a 6 months, hospital-centre exercise program, if they were randomized to EXT.

6.3. Patients Evaluation

All patients underwent evaluation by clinical consult and noninvasive techniques at the following moments (M):

M1 - Baseline evaluation, before the cardiac resynchronizer implant

- Clinical consult: demographic characteristics, clinical functional class (NYHA)
- Symptom-limited cardiopulmonary exercise testing (CPT)
- Echocardiogram
- 24h-Holter with heart rate variability analysis
- Cardiac ^{123}I-MIBG scintigraphy
- EndoPAT
- Blood analysis
- HeartQoL questionnaire

M2 - At 4 months after CRT (3 months after EXT)

- Clinical consult: clinical functional class (NYHA)
- Echocardiogram
- CPT
- Blood analysis

M3 - At 7 months after CRT (6 months after EXT)

- Clinical consult: Clinical functional class (NYHA)
- Symptom-limited cardiopulmonary exercise testing (CPT)
- Echocardiogram
- Cardiac ^{123}I-MIBG scintigraphy
- EndoPAT
- Blood analysis
- HeartQoL questionnaire
- Cardiac events

6.4. Methodology for Dependent Variables Evaluation

6.4.1. Demographic Characteristics

HF patients were assessed at baseline, M1, regarding age, gender, HF etiology and BMI, in clinical consult.

6.4.2. Symptoms severity

Symptoms were classified according to clinical severity, by the clinical functional classification of New York Heart Association, NYHA[490], at baseline, M1, M2 and M3, in clinical consult.

6.4.3. Quality of life

HeartQol Questionnaire from Odridge *et al.*[491] was used for quality of life (Qol) evaluation, allowing determination of quality scores before CRT, M1, and at 7 months after CRT, M3.

This questionnaire[491] was developed as a core heart disease-specific instrument for comparison following interventions such as cardiac rehabilitation, used in more than one heart disease diagnosis. Despite being originally dedicated to ischemic disease, it did include a segment of heart failure patients.

The 14 items in the HeartQoL scale cluster as a bi-dimensional questionnaire with a 10-item HeartQoL physical subscale and a 4-item HeartQoL emotional (psychological) subscale providing a global assessment and evaluation of how much a patient with heart failure perceives, he or she, is bothered by their heart disease. Global score, physical score and emotional score were calculated, knowing that on a HeartQoL scale response of 0–3, higher scores indicate better quality of life. We considered, in this study as, at least, moderate the increase of 0.52, corresponding to 25% of mean value for total HeartQoL score in HF patients, published by Oldridge[491].

6.4.4. Cardiac Scintigraphy with ^{123}I-*meta*-iodobenzylguanidine (^{123}I-MIBG) variables

Cardiac Scintigraphy with ^{123}I-*meta*-iodobenzylguanidine (^{123}I-MIBG) was performed for ANS noninvasive imaging, at baseline, M1, and at 7 months after CRT (6 months after EXT), M3.

Cardiac medication was not suspended, namely beta-blockers, although drugs like antidepressants were stopped for the exam. The imaging protocol typically included anterior and left anterior oblique planar scintigraphic images, obtained by a multiple head gamma camera (*Siemens, model E.Cam),* at 10 to 15 min (early) and at 4 h (late), after intravenous injection of ≈ 185 MBq (adjusted to body weight if necessary) of ^{123}I-MIBG. Thyroid was previously blocked by potassium iodide to inhibit absorption of unbound radioiodine during cardiac imaging.

The acquisition was performed by a nuclear technician at an independent core laboratory (Nuclear Medicine, Quadrantes, Lisbon). A certified nuclear medicine physician and a cardiologist processed and interpreted all images. The physicians and nuclear technician were blinded to randomization for exercise.

Myocardial uptake and distribution were visually assessed in both acquisitions. By measuring ^{123}I-MIBG activity, after drawing regions of interest (ROI) on the heart (H) and mediastinum (M), ^{123}I-MIBG uptake was determined by calculating H/M ratio (HMR), early (HMRe) and late (HMRl). The myocardial wash-out rate (WOR), meaning the rate of ^{123}I-MIBG clearance from myocardium, was calculated as the difference between the early and late H/M and expressed as a percentage of the early H/M[492], as follows:

$$\mathrm{WR} = \frac{HMR_{early} - HMR_{delayed}}{HMR_{early}} \times 100\%$$

This approach is proven to provide a highly reproducible index of cardiac sympathetic activity. By comparing early and late activities, the ^{123}I-MIBG WOR from the myocardium can be derived, providing a parameter that reflects retention of NE by sympathetic neurons[77], since the early HMR reflects the integrity of presynaptic nerve terminals and uptake-1 function and the late HMR combines information on neuronal function from uptake to release through the storage vesicles at the nerve terminals.

In summary, the cardiac sympathetic variables measured were:

- HMRe
- HMRl
- WOR (%).

6.4.5. 24h-Holter monitoring Heart Rate Variability Variables

24h-Holter study *(Burdick, Spacelabs)* was performed before CRT, M1, for detection of cardiac rhythm/arrhythmias, heart rate and for heart rate variability analysis, which corresponds to the beat-to-beat variations in the R-R interval on the ECG, measured over a period ranging from few minutes to 24 hours[87], reflecting the autonomic balance between the sympathetic and parasympathetic pathway action on the intrinsic rhythm of the sinoatrial node of the heart. Spectral analysis of HRV is a widely used method to assess the function of the ANS.

The HRV analysis was only performed before CRT, at M1, because most patients were on pacing rhythm after CRT implant.

Variables used from heart rate variability analysis (domain methods only) were:

- SDNN (Standard deviation of the NN interval);
- SDANN (Standard deviation of the average N-N interval over periods of about 5 minutes);
- RMSDD (Square root of the mean squared differences between adjacent N-N intervals);
- NN50 (Number of adjacent N-N intervals that differ by more than 50 ms).

6.4.6. Cardiopulmonary exercise testing Variables

Exercise functional capacity was evaluated at 3 moments, M1, M2 and M3, by cardiopulmonary exercise test (CPT), which is the best technique to obtain patients maximal and submaximal functional capacity.

Gas exchange analysis is known to provide a highly reproducible measurement of exercise limitation and insights into the differentiation between cardiac or respiratory causes of dyspnea, to assess ventilatory efficiency and to carry prognostic information.

This test was done with the subjects in a non-fasting condition and under the regular medication. A symptom-limited incremental CPT, the modified Bruce protocol (treadmill, 2 warm-up stages, each lasting 3 minutes, first at 1.7 mph and a 0% grade, and second at 1.7 mph and 5% grade), was performed on a treadmill (*MedGraphics CPX Ultima*) with breath-by-breath gas exchange measurements (*Innocor*$_{R,}$ *Innovision,Cardiosolutions*), with online real time calculation of VO_2, CO_{2peak} production, respiratory exchange ratio (RER). Before each test, the gas analyzer was calibrated with gases of known concentrations and the pneumotachograph calibrated before with a known volume (5L syringe).

A twelve-lead ECG (*MedGraphics CPX Ultima*) was recorded continuously and blood pressure (BP) was measured by auscultation, using sphygmomanometer.

Subjects were encouraged to exercise until exhaustion, defined by intolerance, leg fatigue or dyspnea, unless clinical criteria for earlier test termination were observed, trying to achieve RR values superior to 1.1, as indicator of maximal effort, once RR peak is an useful index of peak performance. Patients sat on a chair as soon as they stop walking, while recovery measurements were taken. BP was recorded at baseline, during the 2nd minute of each stage, at peak exercise and during recovery. VO_{2peak} was considered the highest attained VO_2 during the final 30s of exercise and ventilatory anaerobic threshold (VAT) was estimated with the V-slope method, registering time to VAT[493]. HR recovery (HRR), a simple marker of parasympathetic activity, was calculated as the difference between peak HR and HR at one minute after exercise cessation[95]. The recovery period was maintained until 7 minutes after peak effort.

CPT Variables measured were the following:

- CPT dur (Cardiopulmonary Testing duration; seconds);
- VAT time (Time to Ventilatory Anaerobic Threshold; seconds);
- VO_{2p} (Peak oxygen consumption; ml/kg/min);
- VE/VCO_2 slope (Minute ventilation – carbon dioxide production relation);
- HRbas (Baseline heart rate; beats per minute) and HRmax (Maximal heart rate; beats per minute);
- HRR1, HRR6 (Heart rate recovery at 1st minute and 6th minute; seconds)
- SBPbas and SBPmax (Baseline and Maximal Blood Pressure; mm Hg)

6.4.7. Echocardiography Variables

Cardiac function was studied by echocardiography. A rest transthoracic echocardiogram, bidimensional, Doppler, Tissue Doppler Imaging (TDI) and strain analysis by speckle tracking, was performed, with an ultrasound machine (*General Electric, GE Vivid 9*), at the 3 moments. The exam was done by the Echocardiography laboratory cardiologists, who were blinded to experimental protocol and group randomization. The usual measurements of left ventricular systolic and diastolic function and right ventricular systolic function were undertaken, according to recommendations[494]. The calculation of left ventricular volumes (LVED Vol, LVES Vol) and left ventricular ejection fraction (LVEF) was performed by Simpson`s method. Global longitudinal strain of left ventricle (GLS) was determined by using speckle tracking. Tissue Doppler analysis of mitral annulus motion was done for diastolic function evaluation.

Evaluated echocardiographic variables were:

- LVEF (Left ventricular ejection fraction; ml)
- LVEDVol (Left ventricular end diastolic volume; ml)
- LVESVol (Left ventricular end systolic volume; ml)
- LVM (Left ventricular mass; grams)
- GLS (Global longitudinal strain; %)
- E/e' (Ratio between E wave from mitral inflow and e' wave from mean mitral annular motion, TDI)
- TAPSE (Tricuspid annular plane systolic excursion; mm)
- LAVol (Left atrial volume; ml)
- RAVol (Right atrial volume; ml)
- PSAP (Pulmonary Systolic artery pressure; mm Hg)

Images were acquired by 3 experient echocardiographists and all measurements were revised by a single echocardiographist.

LVEF, LV volumes and GLS measurements were performed at least 3 times, for obtaining an average, excluding discrepant results.

6.4.8. EndoPAT

Endothelial function study was undertaken by evaluating digital arterial elasticity, by EndoPAT (*Itamar Medical, Israel*). It detects plethysmographic pressure changes in the finger tips caused by the arterial pulse and translates these to peripheral arterial tone (PAT). Endothelium-mediated changes in vascular tone after occlusion of the brachial artery reflect a downstream hyperemic response, reactive hyperemia, which is a measure for arterial endothelial function[496].

The EndoPAT (peripheral artery tonometry) was done to each patient at M1, M2 and M3 conducting to pre and post-occlusion values, according to the protocol[497].

Many variables are known to acutely influence endothelial function and they were controlled for the testing. Alcohol and caffeine were limited in the 48 hours prior to testing. The protocol consisted in inflating a standard blood pressure cuff , eliciting a 5-minutes occlusion of the brachial artery, in a fasting state patient who had been lied down for at least 5 minutes in a quiet environment, mild temperature. The cuff was then released and consequently the surge of blood flow caused an endothelium-dependent vasodilation, called Flow Mediated Dilatation (FMD). Measurements on the contralateral arm were used to control for concurrent nonendothelium-dependent changes in vascular tone. The dilatation, manifested as reactive hyperemia, is captured by EndoPAT™, as an increase in the PAT Signal amplitude, which is diminished in endothelial dysfunction. A post-occlusion to pre-occlusion ratio is calculated by the EndoPAT™ software, providing the EndoPAT™ index, RHI (reactive hyperemia index).

In addition, the EndoPAT provides a measure for arterial stiffness: the augmentation index (AI). AI can be adjusted for a HR of 75 (AI@75).

Endothelial Study Variables determined were:

- Reactive Hyperemia index - peripheral arterial tonus (RHI-PAT)
- Augmentation index (AI, AI@75)

6.4.9. Blood Analysis

Analytic parameters were obtained for inflammation, apoptosis, endothelial and cardiac function were obtained from blood analysis at M1, M2 and M3.

The variables analysed were the following:

- TNF-α (Tumor necrotic factor- alpha; pg/ml)
- IL-6 (Interleukin-6; pg/ml)
- sCD40 (soluble Cluster of differentiation-40; pg/ml)
- sFasL (soluble Protein Fas ligand; ng/ml)
- hs-CRP (high sensitivity C Reactive Protein; mg/L)
- BNP (Brain Natriuretic Peptide; pg/ml)
- NO (Nitric Oxide; µmol/L)

NO determination (µmol/L) was performed in Universidade Nova de Lisboa, according to the usual method[498] after previous blood collection and storage at – 80 C ^{O}, in Hospital Santa Marta. Blood sample preparation for NO determination was carefully treated, not to artifactually create NO products or metabolites during sample preparation. Blood was centriphugated and plasma separated and frozen. Posteriorly, it was transported for measurement of NO level by quimioimunoluscence method by 280i Nitric Oxide Analyzer (*NOA^{TM} ,Sievers Instruments*).

TNF-α (pg/ml), IL-6 (pg/ml), sFasL (ng/ml) and sCD40 (pg/ml) were measured, according the usual method Elisa[499] at Instituto de Medicina Molecular e Terapêutica (IMMT) and blood was collected in Hospital Santa Marta. Blood was centriphugated and plasma was frozen at -80º C. Transport was conducted in adequate conditions, with dry ice, to IMMT.

BNP (pg/ml), CRP (mg/L) were collected in Hospital Santa Marta and analysed, according to usual method at Hospital S. José Laboratory.

Commercial high-sensitive enzyme-linked immunoassay kits (*ELISA kits R&D Systems*) were used to determine the concentration of BNP, hs-RCP, IL-6, TNF-α, sCD-40 and sFasL in the patients serum.

6.4.10. Cardiac events

Cardiac events, determined at M3 and evaluated by consult, telephone interview, and hospital registries, were defined as: all-cause mortality, cardiac mortality, cardiac hospitalization (heart failure, severe arrhythmia, cardiac arrest) and evaluated separately and combined.

6.5. Implantation CRT Protocol

Implantation was performed in Hospital Santa Marta, according to the protocol for standard biventricular pacing implantation techniques[500]. The CRT or CRT-D is a small equipment, which includes a generator and three leads, used to correct ventricular dyssynchrony. Implantation of all system is performed under local anesthesia with standard ECG monitoring and pulse oximetry, under fluoroscopic guidance, by a dedicated intervention team consisting of two electrophysiologists, a technician and a nurse. An active fixation lead is positioned via the left cephalic venous access, in an apical or septal position, with a second lead inserted through an introducer sheath into the left subclavian vein and implanted at the high right atrium. Specific long delivery systems and lead shapes allow to cannulate the coronary sinus using a long sheath via the left subclavian vein and, thereby, after performing an angiography obtained with a small amount of nonionic contrast material during balloon occlusion, ensure progression of a transvenous left ventricular over-the-guide wire lead to be positioned in a distal target position (lateral median, postero-lateral or antero-lateral cardiac vein). If the catheter cannot be located into the coronary sinus easily, it is changed by another catheter having a different shape or an electrophysiology catheter designed to cannulate the coronary sinus. After positioning the left ventricular lead to its final location, the

guiding catheter is withdrawn. The procedure is performed using left anterior oblique, right anterior oblique and posteroanterior views. After positioning the atrial and ventricular leads, the capture thresholds testing are performed until obtaining a good safety margin. Generators are placed subcutaneous in the left pectoral region. Optimal programming includes atrio-ventricular and sequential ventricular timings, chronotropic response, output, sensitivity and therapies (ATP and shocks) delivery in tachycardia zones. All patients perform a thoracic X-Ray and an echocardiogram before discharge.

6.6. Protocol of Exercise Training

Patients from the EXT group were submitted, after careful functional evaluation, to a supervised, controlled, ECG monitored, based-hospital exercise program, twice a week, 60 minutes sessions, on non-consecutive days, during 6 months (48 sessions), including aerobic high intensity interval training (HIIT) , resistance and sensoriomotor exercises.

Patients were stimulated to increase physical activity at home, especially in the days without training session.

Aerobic interval high intensity training (HIIT) method was selected for the development of cardiorespiratory system.

The EXT design was based on Wisloff study[445], using an interval exercise training method, because of Wisloff good cardiovascular results.

The program began with the warm-up and aerobic training, by using treadmill walking. Patient warm-up lasted for 10 minutes at 50% to 60% of HRmax (obtained in the CPT), before walking to 4 intervals of 2 minutes at 90 to 95% of HRmax. Aerobic exercise training was initiated with shorter aerobic intervals and, only at the second month, the Wisloff protocol[445] was started. The HIIT protocol comprised 4 interval training periods (high intensity) and 3 active pauses (moderate intensity) between interval training periods. Each interval, including the last one, was separated by 2 minutes active pauses, walking at 60% to 70% of HRmax (Fig.2).

After the first month, every week, each interval training and active pause was increased by 30 seconds, until arriving to the 4 minutes work with 3 minutes of active rest, at the end of the second month. By the end of the progression, the protocol includes 28 minutes of aerobic HIIT, maintained to the end of EXT intervention period.

The moment at 3 months, M2, intended to provid accurate information for updating the exercise prescription, allowing to adjust aerobic training intensity, as the target heart rate (THR) is adjusted. The speed and inclination of the treadmill should be continuously adjusted to ensure that, in each interval, THR would be respected, throughout the aerobic training period.

Compared to continuous exercise training, this interval method allows HF patients to complete short periods of exercise at high intensity (which stress heart's ability),

without deleterious effects of undue stress and fatigue. However, due to our patients frequent previous severe clinical status and physical deconditioning, a slower exercise prescription progression was chosen in the first 1-2 months, whenever necessary. The long intervention duration of 6-month allowed to use initially a continuous moderated exercise training protocol, passing afterwards to interval training.

Another difference in this EXT program protocol was the incorporation of resistive and sensoriomotor exercises. Muscle resistance training consisted of 1-2 sets of 8-12 repetitions, for each of the 6 exercises. The patients were instructed regarding correct exercise techniques and avoidance of the Valsalva manoeuver.

Sensoriomotor training included 1 to 2 exercises, lasting 40 seconds each, with 3 repetitions with 20 seconds rest between sets. The resistance and sensoriomotor training period lasted 15-17 minutes.

These non aerobic exercises were aimed to improve muscle strength and coordination in movements, which are frequently lacking in HF patients, and consequently to enhance the performance of muscles not involved in the aerobic mode of exercise, influencing positively daily life activities and consequently quality of life. The inclusion of resistance and sensoriomotor exercises in the program is fundamental for complementing the effects of aerobic exercise in HF patients.

Every session ended with a 5-7 minutes cool-down, consisting on stretching exercises and relaxation.

All patients were monitored, using a 12-lead ECG, during the aerobic training to control both exercise intensity and safety during the high intensity workout, and a heart rate monitor (*Polar, Electro, Kempele, Finland*), during the execution of the other exercises. Blood pressure was monitored and the Borg 6-to-20 scale was used to assess the rate of perceived exertion during and after each training session.

The exercise schedule was set according to the patient possibility and maintained during the 6-month intervention. No more than 2 patients were scheduled at the same time of the day.

EXT protocol used in this study**,** schematically displayed in the fig.2, consisted of:

- Warm-up – 10', 60-70% HR max;
- Resistance Training- 2x (8-12) repetitions;

- Aerobic – 4x4'@90-95% HR max; 3x3'@50-70%HR max;
- Cool-down- 5-7', HR reduction, relaxation, stretching.

The progression of exercise, along time, was as follows:

- 1 Month - Aerobic continuous 60-70% HR max;
- 2-3 Months – 4x2' 90-95% HR max; 3X2'@50-70% HR max; increase of 30'' 15/15 days;
- 4-6 Months – 4x4'@90-95% HR max ; 3x3'@50-70%HR max.

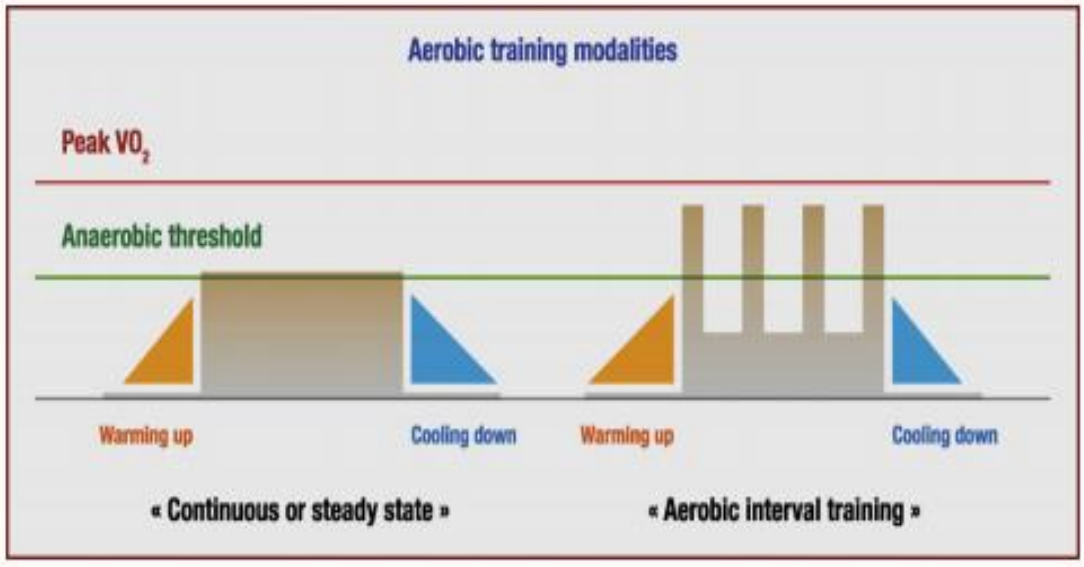

Fig.2. Exercise training modalities with graphicaly displayed VO_{2P} variation along time: HIIT (high intensity interval training) protocol, on the right, and MICE (moderate intensity continuous exercise) protocol, on the left.

6.7. Definition of CRT responder

Once there are several definitions for CRT response in different studies, as previously discussed in a previous chapter , we considered appropriate to specify the definitions used in this study.

Clinical responder was defined as the patient who increases at least one functional class of NYHA.

Echocardiographic responder was defined as the patient who increases at least 5% (absolute value) of LVEF.

Functional responder was defined as the patient who increases at least 1ml/kg/min VO_{2p}.

6.8. Data base and Statistics

Exploratory analysis was carried out for all variables, tested for normality and homogeneity of variance with the Shapiro-Wilk and Levene's tests, respectively. Categorical data were presented as frequencies and percentages, and continuous variables as mean or median, standard deviation (SD) or inter-quartile range (IQR, 25^{th} percentile-75^{th} percentile), as appropriate. Chi-square, Fisher's exact tests, parametric t-test, paired and unpaired and non-parametric Wilcoxon and Mann-Whitney were used, as adequate. The multivariable analysis was performed using logistic regression models; all the variables with a p-value <0.15 in the univariable analysis were considered.

Hosmer-Lemeshow goodness-of-fit test was used, with high p-value indicating that the model is performing well. Box-Tidwell transformation to test the assumption of linearity in the logit of continuous variables was used. The 95% confidence intervals (CI) were also calculated, as required. The level of significance α=0.05 was considered.All data were analyzed using SPSS 22.0 (*IBM Corp. Released 2013. IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp*).

7. RESULTS

Results were organized as follows:

- Effects of Exercise Training Intervention after CRT: A randomized controlled trial (7.1)
- Effects of CRT with or without Exercise: An observational study (7.2)

7.1. Effects of Exercise Training Intervention after CRT: a Randomized Controlled Trial

7.1.1. Randomized Groups characterization

From the sample of 121 HF patients selected for CRT implant, 62 had criteria for inclusion in the controlled randomized trial and initially agreed to be randomized for exercise or control and to participate in the study.

34 patients were randomized for exercise, although afterwards 12 P reffused to enter the exercise program, for financial reasons (8 P), transportation difficulties (3 P) and professional unavailability (1P). The remaining 22 patients in the EXTG completed it, with no drop-out. 28 P were randomized for the control group (CG).

Despite the reffusal of some patients to perform exercise, patients submitted to randomization, in the 2 groups, confirmed to be statistically identical (EXTG and CG), regarding the characeristics of randomization (table 1A).

Table 1A. Demographic and clinical characteristics of randomized patients

	Exercise Group (EXTG)	Control Group (CG)	P
N	22	28	
Age (years)	67.5±9.8	66.7±10.8	Ns
Female (n, %)	5 (22.7%)	7 (25%)	Ns
BMI (Kg/m^2)	27.18±3	27.54±3.5	Ns
NYHA II-III (n, %)	21 (95%)	28 (100%)	Ns
NYHA IV (n, %)	1 (5%)	0 (0%)	Ns
LVEF (%)	26.68±6.21	26.18±8.24	Ns
LVEF<20%	3 (13.6%)	9 (32.1%)	Ns
AF (n, %)	9 (40.9%)	11 (39.2%)	Ns
Ischemic DCM (n, %)	9 (40.9%)	10 (35.7%)	Ns
wQRS (msec)	157.5±21.2	146.7±46.2	Ns

BMI- body mass índex (kg/m^2); NYHA-New York Heart Association clinical classification(n-number,%); LVEF- left ventricular ejection fraction; AF –atrial fibrillation; DCM – dilated cardiomyopathy; Wqrs – QRS lenght

The results of this clinical trial, evaluating 50 randomized patients, are displayed in the next sections:

- Baseline values for dependent variables (7.1.2.)
- Variation for dependent variables after intervention (7.1.3)

7.1.2. Baseline Dependent Variables

Values for dependent variables (expressed as median and interquartile range, 25th percentile-75th percentile), collected at M1, baseline, previous to CRT, in the randomized patients, are displayed in table 1B.

Tabela 1B. Baseline Dependent Variables in Exercise (EXTG) and Control (CG) groups

Variables	EXTG (n=22)		CG (n=28)		
	Median	P_{25}-P_{75} (min-max)	Median	P_{25}-P_{75} (min-max)	P
NYHA class	3	2.66-3.07 (2-4)	3	2.71-3 (2-3)	Ns
HQoL T score	0.64	0.56-1 (0.28-1.57)	0.78	0.555-1.217 (0.01-2.5)	Ns
HQoL Ph score	0.4	0.31-0.74 (0-1.2)	0.4	0.327-0.935 (0.01-2.4)	Ns
HQoL Ps score	1.25	0.9-1.59 (0-2.5)	1.33	0.929-1.736 (0.01-3)	Ns
LVEF (%)	26.5	23.93-29.44 (12-34)	29	22.98-29.37 (11-42)	Ns
LVEDVol (ml)	203	178.54-250.91 (98-433)	195	183.47-248.41 (104-420)	Ns
LVESVol (ml)	139	124.66-179.8 (49-300)	155	132.83-189.94 (78-322)	Ns
LVMass (g)	297	288.17-364.07 (224.05-583.79)	313.21	295.963-373.941 (169-517.69)	Ns
GLS (%)	-5.5	-7.41-(-3.22) (-13-0.1)	-7	-7.68-(-5.14) (-10-0)	Ns
E/e'	15	13.6-29.2 (7-50)	19	15.42-27.51 (6-46)	Ns
LAVol (ml)	74	58.88-84.75 (38-102)	67	62.55-119.34 (22-222)	Ns
RAVol (ml)	43	28.17-63.03 (13-89)	37	19.33-76.31 (12-254)	Ns
TAPSE (mm)	17	14.75-20.9 (9-30)	17	15.61-19.91 (10-31)	Ns
PSAP (mm Hg)	40	34.15-47.71 (29-70)	40	38.71-49.08 (28-71)	Ns
HRbas (bpm)	75	69.47-84.74 (51-107)	75.5	71.04-85.76 (57-116)	Ns
HRmax (bpm)	111	107.56-131.91 (82-173)	122	114.61-135.48 (72-160)	Ns
SBPbas (mm Hg)	110	104.66-120.24 (90-140)	120	113.55-129.79 (90-150)	Ns
SBPmax (mm Hg)	139	130-147.2 (120-170)	140	128-156 (120-178)	Ns
HRR1 (bpm)	11	8-14.5 (4-19)	16	10-20 (8-22)	Ns
HRR6 (bpm)	31	25-38 (15-48)	42	28-49 (12-54)	Ns
CPTdur (sec)	360	277.32-491.23 (57-747)	327.5	264.23-518.77 (47-900)	Ns
VATtime (sec)	180	161.64-313.5 (90-510)	279	151.80-407.8 (90-540)	Ns
VO_{2p} ml/kg/min)	13	11.99-16.34 (9-21)	13	11.79-18.52 (6-32)	Ns
$VEVCO_2$slope	31	28.84-39.29 (22-56)	37	32.14-42 (22-57)	Ns
HMRe	1.46	1.314-1.656 (1-2)	1.45	1.269-1.578 (1-2)	Ns

HMRl	1.28	1.169-1.468 (1-2)	1	1.31-1.38 (1-2)	Ns
WOR (%)	50.5	44.56-58.2 (20-90)	44.73	42.236-54.357 (20-79)	Ns
RHI	1.53	1.19-1.7 (1-2)	1.5	1.25-1.86 (1-3)	Ns
AI (%)	2	-10.6-4.79 (-27-26)	-3	-24.72-13.14 (-125-58)	Ns
AI@75 (%)	-8	-14.51-0.7 (-37-18)	-7	-15.07-7.6 (-42-52)	Ns
wQRS	150	141.27-173.24 (130-200)	120	117.47-154.53 (120-200)	Ns
SDNN	105.5	85.59-167.91 (42-344)	109	79.88-191.96 (53-347)	Ns
SDANN	76	56.57-107.43 (0.1-159)	83	55.96-122.4 (35-204)	Ns
RMSDD	42	14.73-106.2 (12-345)	64	28.36-145.04 (12-244)	Ns
pNN50	16	3.05-29.35 (0.1-84)	21	8.25-46.30 (1-78)	Ns
BNP (pg/ml)	292	224.11-634.41 (42-1868)	254	301.26-811.12 (89-2340)	Ns
hs-CRP (mg/L)	2	1.56-6.35 (0-20)	2	0.09-13.37 (0.001-86)	Ns
TNF-α (pg/ml)	2	1.94-3.96 (1-10)	2	1.87-3.13 (1-6)	Ns
IL-6 (pg/ml)	7.5	3.85-13.35 (2-24)	7	4.28-9.72 (1-18)	Ns
sCD-40 (pg/ml)	3494	2556.03-5874.77 (528-9207)	3459	2693.68-6171.24 (1200-10235)	Ns
sFasL (ng/ml)	55	44.31-73.22 (26-126)	57	45.22-67.50 (21-130)	Ns
NO (µmol/L)	53	35.43-61.13 (11-105)	48	41.23-66.43 (20-126)	Ns

All values are expressed as median, inter-quartile range 25^{th} percentile-75^{th} percentile (P_{25}-P_{75}), minimal and maximal values. NYHA - New York Heart Association functional clinical classification; HQoL T- total score of quality of life questionnaire HeartQoL; HQoL Ph – Physical score of quality of life questionnaire HeartQoL; HQoL Ps – psychologic score of quality of life of HeartQoL; LVEF – left ventricular ejection fraction; LVEDVol – left ventricular end-diastolic volume ; LVESVol – left ventricular end-systolic volume; LVMass – left ventricular mass; GLS – left ventricular Global Longitudinal Strain; E/e'- ratio between E wave from pulsed Doppler left ventricular inflow wave and tissue Doppler mitral annular mean e'; LAVol – left atrial volume; RAVol – right atrial volume; TAPSE – Tricuspide Annular Plane Systolic Excursion; PSAP – pulmonary arterial systolic pressure; HRbas – baseline heart rate; HRmax – maximal heart rate; SBPbas – baseline systolic pressure; SBPmax – maximal systolic pressure; HRR1 – heart rate recovery at 1^{st} min; HHR6 – heart rate recovery at 6^{th} min; CPTdur – duration of cardiopulmonary testing; VATtime – time to ventilatory anaerobic threshold; VO_{2P} – ventilatory oxygen in consumption;$VEVCO_2$ slope – slope of ventilatory exchange of carbon dioxide;HMRe – ^{123}I-MIBG heart to mediastinum ratio WOR - wash-out ratio; RHI – reactive hyperemia index; AT – augmentation index; AT@75 – augmentation index corrected for average heart rate of 75 bpm;Wqrs – QRS length; SDNN – standard deviation of NN interval; SDANN – standard deviation of the average N-N interval over periods of about 5 minute;RMSDD- square root of the mean squared differences between adjacent N-N intervals, NN50 – number of adjacent N-N intervals that differ more than 50 ms; BNP – plasmatic brain natriuretic peptide; CRP –plasmatic C reactive protein; TNF-α – plasmatic tumor necrotic factor alpha; IL-6 – plasmatic interleukin 6; sFasL - plasmatic soluble FasL; sCD-40 – plasmatic soluble citokyne 40;NO – plasmatic nitric oxide

7.1.3. Variation of Dependent Variables after Exercise Intervention in CRT-HF patients

Values for modification of dependent variables after exercise intervention post-CRT in EXTG and after CRT only in CG are shown in the next tables (table 3-table 9).

7.1.3.1. Symptoms and quality of life effects of Exercise Intervention

Clinical functional class improved in both groups, exercise and control, although more in the exercise group, especialy attaining significance at 6 months of exercise program, as observed in table 3.

At 3 months of EXT there were 90.1% clinical responders in EXTG vs 82.6% in CG.

At 6 months, clinical responders increased in EXTG to 95% vs 78.5% in CG.

Quality of life, evaluated by HeartQol questionnaire, which had baseline low scores, improved at least moderately (more than 0.5 in total score) in 95% of patients in EXTG and 96% in CG. One only patient did not improve HeartQoL score at all, from the CG.

Quality of life scores improved significantly, in all dimensions, in both exercise and control group patients, with significant variation (p=0.000), but without statistical difference in randomized groups, as shown in the table 4.

Table 3. NYHA Clinical Functional Class variation after CRT with Exercise (EXTG) and without exercise (CG)

NYHA Δ	EXTG			CG			EXT/ CG
	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	P	P
NYHA M2-M1	-1.27	-1.6- (-0.94)	0.000	-0.95	-1.2-(-0.71)	0.000	0.094
NYHA M3-M1	-1.52	-1.82-(-1.23)	0.000	-1.00	-1.34- (-0.65)	0.000	0.034

All values are expressed as median, inter-quartile range 25^{th} percentile-75^{th} percentile (P_{25}-P_{75}).
NYHA – New York Heart Association clinical classification; M2-M1 - variation between moment 2 and 1; M3-M1 – variation between moment 3 and moment 1; EXTG – exercise training group; CG – control group

Table 4. Quality of Life score variation after CRT with Exercise (EXTG) and without exercise (CG)

HQol Δ	EXTG			CG			EXT/ CG
	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	P	P
HQol T M3-M1	1.26	0.84-1.68	0.000	0.94	0.50-1.38	0.000	0.246
HQol Ps M3-M1	1.05	0.71-1.39	0.000	0.789	0.29-1.28	0.000	0.223
HQol Ph M3-M1	1.52	1.23-1.82	0.000	1.211	0.79-1.62	0.000	0.146

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}.P_{75}).
HQoL T – total score of quality of life questionnaire HeartQol (Oldridge); HQol Ph – physical score of quality of life questionnaire HeartQol; HQol Ps – psychologic score of quality of life questionnaire HeartQol; EXTG – exercise training group; CG – control group

7.1.3.2. Echocardiographic effects of Exercise Intervention

LVEF, increased significantly in both randomized groups, more at 6 months, without significant difference.

At 3 months, 68.4 % echocardiographic CRT responders in the exercise group and 75 % in the control group were present, but at 6 months responders increased to 81.8 % in the exercise group and decreased to 72.7 % in the control group.

LVED volume decreased in both groups, significantly only at 6 months.

LVES volume decreased also in both groups, significantly at 6 months. At 3 months LVES volume decrease was significant only in CG.

LV mass decreased nonsignificantly in both randomized groups, but especially at 6 months with a tendency for significance (p=0.094), only in the group of exercise (variation of 38 g EXTG vs 11 g CG).

E/e', decreased in both subgroups, however with statistical significance only at 6 months for EXTG (p=0.042).

Regarding the variation of all other echocardiographic parameters, no statistical significance was obtained.

Echocardiographic parameters variation are displayed in table 5.

Table 5. Echocardiographic variables variation after CRT with Exercise (EXTG) and without exercise (CG)

Echo Δ	EXTG			CG			EXT/CG
	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	P	P
LVEF M2-M1	11.90	5.29-18.50	0.004	9.56	5.67-13.45	0.000	0.545
LVEF M3-M1	11.86	6.66-17.06	0.001	10.41	4.69-16.12	0.003	0.734
LVEDVol M2-M1	-8.79	-36.99-19.42	0.594	-18.39	-46.13- 9.35	0.218	0.750
LVEDVol M3-M1	-21.71	-42.38-(-1.05)	0.019	-14.23	-28.77- 0.31	0.042	0.692
LVESVol M2-M1	-24.0	-53.0-5.0	0.140	-28.78	-53.50-(-4.05)	0.023	0.866
LVESVol M3-M1	-22.24	-38.80- (-5.68)	0.015	-26.36	-43.38; -9.34	0.005	0.834
LV Mass M2-M1	-7.37	-74.68-59.93	0.972	-3.31	-37.22-30.59	0.831	0.805
LV Mass M3-M1	-38.58	-82.63-5.48	0.094	-11.90	-48.39-24.58	0.355	0.425
GLS M2-M1	-0.75	-4.15-2.65	0.528	-0.5	-3.22-2.22	0.391	0.878
GLS M3-M1	-2.36	-5.53-0.81	0.109	-0.69	-2.69-1.30	0.504	0.331
E/e' M2-M1	-0.88	-5.42-3.67	0.599	-5.17	-20.29-9.96	0.345	0.414
E/e' M3-M1	-6.40	-12.28-(-0.52)	0.042	-4.3	-9.47-0.87	0.097	0.765
LAVol M2-M1	3.33	-29.02-35.69	0.917	-21.63	-47.52-4.27	0.089	0.181
LAVol M3-M1	-9.14	-33.86; 15.58	0.499	6.2	-27.27-39.67	0.685	0.475
RAVol M2-M1	-15.67	-37.94-6.60	0.074	-6.00	-22.21-10.21	0.326	0.282
RAVol M3-M1	-11.71	-30.43- 7.00	0.150	-6.00	-25.22-13.22	0.441	0.606
TAPSE M2-M1	1.67	-1.41-4.75	0.167	0.79	-2.35- 3.93	0.552	0.667
TAPSE M3-M1	0.50	-2.42-3.42	0.893	-1.31	-4.00-1.39	0.385	0.402
PSAP M2-M1	-2.00	-13.75-9.75	0.858	-3.27	-9.03-2.49	0.271	0.640
PSAP M3-M1	-2.29	-16.11-11.54	0.400	-4.82	-11.35-1.72	0.130	0.328

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}).
LVEF – left ventricular ejection fraction (%);LVEDVol – left ventricular end-diastolic volume (ml);LVESVol – left ventricular end-systolic volume (ml); LVMass – left ventricular mass (g); GLS – left ventricular Global Longitudinal Strain (%); E/e'- ratio between E wave from pulsed Doppler left ventricular inflow wave and tissue Doppler mitral annular and mean e';LAVol – left atrial volume (ml); RAVol – right atrial volume (ml); TAPSE – tricuspide annular plane systolic excursion (mm); PSAP – pulmonary systolic artery pressure (mm Hg)

7.1.3.3. Exercise functional capacity effects of Exercise Intervention

From CPT variables, CPT duration showed significant increase, only in the exercise group, with a significant difference regarding those who did not exercise in CG, especially at 6 months (p=0.002). VAT increased significantly in the exercise group, at 3 and especially at 6 months, while in the control group increased less and only at 6 months. There was a significant difference at 3 months (p= 0.006) and a tendency for a difference at 6 months (p=0.06), between the 2 randomized groups.

VO_{2p} increased in both groups, but statistically significantly only at 3 months (p=0.026) in EXTG (EXTG 2.13 ml/kg/min vs CG 0.25ml/kg/min; p=ns).

Functional response (defined as those who increased VO_{2p} at least 1 ml/kg/min), at 3 months, occurred in EXTG in 77.2% patients vs 53.8% in CG and at 6 months, in 77.2% in EXTG vs CG in 53.8%.

The variation of HRR1, at 3 months, was significantly different between both groups, better only for CG. At 6 months, HRR1 improves in both, but significantly only in CG.

All other variables variation had no significant difference, as shown in table 6.

Table 6. Exercise Testing variables variation after CRT with Exercise (EXTG) and without exercise (CG)

CPET Δ	EXTG			CG			EXT/ CG
	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	P	P
HR bas M2-M1	1.56	-7.07-10.19	0.469	-0.19	-8.86- 8.48	0.959	0.696
HR bas M3-M1	-4.47	-12.95- 4.01	0.443	-2.64	-12.10- 6.81	0.541	0.891
HR max M2-M1	0.88	-11.66-13.41	0.552	5.77	-3.30- 14.83	0.218	0.683
HR max M3-M1	-3.59	-16.72-9.54	0.795	-6.40	-20.59- 7.79	0.551	0.655
HRR1 M2-M1	6.5	-2.62-15.62	0.079	-5.93	-14.48-2.61	0.182	0.024
HRR1 M3-M1	-2.19	-12.73-8.35	0.979	-15.46	-29.35-(-1.58)	0.039	0.110
HRR6 M2-M1	2.46	-3.67-8.60	0.25	-1.54	-11.87-8.79	0.861	0.39
HRR6 M3-M1	-1.76	-9.76-6.23	0.76	-7.31	-15.39-0.77	0.140	0.26
SBP bas M2-M1	10.56	1.10-20.02	0.033	-0.59	-12.75-11.57	0.819	0.127
SBP bas M3-M1	6.33	-2.82-15.49	0.141	-1.13	-12.23-9.96	0.972	0.259
SBPmax M2-M1	12.81	-2.32; 27.95	0.093	30.71	-9.18-23.55	0.375	0.669
SBPmax M3-M1	12.78	2.36-23.20	0.034	3.21	-11.58-18.01	0.755	0.220
CPTdur M2-M1	167.69	38.9-296.47	0.017	77.2	-40.66-195.06	0.256	0.202
CPTdur M3-M1	235.13	83.07- 387.18	0.008	24.00	-50.42- 98.42	0.397	0.002
VATtime M2-M1	174.64	86.88-262.39	0.006	-6.86	-116.79-103.08	0.612	0.006
VATtime M3-M1	216.42	109.7-323.14	0.004	50.00	-116.12-216.12	0.028	0.064
VO2p M2-M1	2.44	-0.05-4.92	0.026	2.20	-1.67-6.07	0.285	0.545
VO2p M3-M1	2.18	-0.53-4.80	0.080	0.25	-2.59-3.09	0.893	0.277
Ve/VCO2sl M2-M1	-3.62	-9.64-2.41	0.294	-7.67	-12.50-(-2.83)	0.014	0.152
Ve/VCO2sl M3-M1	0.08	-4.88-5.03	0.551	-2.63	-12.36-7.11	0.483	0.750

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}).
HRbas – baseline heart rate (bpm); HRmax – maximal heart rate (bpm); SBPbas – baseline systolic pressure (mm Hg); SBPmax – maximal systolic pressure (mm Hg); HRR1 – heart rate recovery at 1st min; HHR6 – heart rate recovery at 6th min (bpm), CPTdur – duration of cardiopulmonary testing (sec), VATtime – time to ventilatory anaerobic threshold (sec), VO_{2P} – ventilatory oxygen consumption (ml/kg/min); $VEVCO_2$ slope – minute ventilation-carbon dioxide production relation slope; EXTG- exercise training group; CG – control group;

7.1.3.4. Imaging ANS function effects of Exercise Intervention

In randomized groups, variations of the parameters of ANS function, HMRe, HMRl and WOR, in EXTG and CG groups were not significant, as observed in table 7.

Table 7. Scintigraphic variables variation after CRT with Exercise (EXTG) and without exercise (CG)

^{123}I-MIBG Δ	EXTG			CG			EXTG/CG
	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	P	P
HMRe M3-M1	-0.04	-0.42-0.21	0.508	0.05	-0.13-0.22	0.722	0.554
HMRl M3-M1	-0.02	-0.3-0.23	0.937	-0.01	-0.15-0.14	0.760	0.651
WOR M3-M1	-3.34	-48.13- 34.16	0.642	4.46	-3.48-12.41	0.330	0.617

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}).
HMRe – ^{123}I-MIBG early heart to mediastinum ratio; ^{123}I-MIBG HMRl – late heart to mediastinum ratio; WOR - ^{123}I-MIBG wash-out rate; EXTG – Exercise Training group; CG – Control group

7.1.3.5. Endothelial effects of Exercise Intervention

Endothelial function and arterial stiffness parameters from peripheral arterial tonometry, RHI, AI and AI@75 did not vary significantly in EXTG and CG. Also, NO did not significantly change, as displayed in table 8.

Table 8. Endothelial function variables variation after CRT with Exercise (EXTG) and without exercise (CG)

Endopat and NOΔ	EXTG			CG			EXT/ CG
	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	p	P
RHI M3-M1	0.20	-0.17-0.57	0.257	-0.07	-0.56-0.42	0.763	0.412
AI M3-M1	8.94	-2.16-20.04	0.107	13.12	-11.52-37.89	0.423	0.631
AI@75 M3-M1	8.24	-2.16-18.63	0.107	9.62	-1.21- 20.46	0.103	0.986
NO M3-M1	7.66	-27.71-43.03	0.657	2.75	-16.62-22.13	0.616	0.857

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}), minimal and maximal values.
RHI – Reactive Hyperemia Index; AI – Augmentation Index (%); AI@75 – Augmentation Index adjusted for average heart rate of 75 bpm (%); NO – Nitric oxide (μmol/L); EXTG – Exercise training group; CG – Control group

7.1.3.6. Inflammatory and Apoptosis effects of Exercise Intervention

TNF-α showed a significant decrease only in patients submitted to exercise trainning (p=0.016) with a significant difference regarding the small nonsignificant difference in control group (p=0.008).

BNP decreased in both groups with a tendency for significance in EXTG and CG (p=0.09 and 0.06, respectively), without significant difference between the groups.

Other analysed blood markers, including hs- CRP sCD-40, sFasL and IL-6, did not change significantly in the randomized groups**.**

sCD-40, sFasL and BNP were determined only at M1 and M3 for technical reasons.

Values are shown in table 9.

Table 9. Biomarkers variation after CRT with Exercise (EXTG) and without exercise (CG)

Biomarkers Δ	EXTG			CG			EXTG/CG
	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	P	P
TNF-α M2-M1	0.18	-1.92-2.28	0.439	-0.43	-1.24- 0.38	0.298	0.936
TNF-α M3-M1	-1.08	-1.84-(-0.32)	0.016	0.12	-0.19-0.43	0.414	0.008
IL-6 M2-M1	0.5	-5.85-6.85	0.317	2.00	-6.65-10.65	0.416	0.857
IL-6 M3-M1	-0.33	-7.65-6.99	0.684	-3.13	-7.91-1.66	0.127	0.755
sFasL M3-M1	5.29	-14.71-25.28	0.310	4.00	-4.74-12.74	0.414	0.328
sCD40 M3-M1	3753	-9885-17391	0.180	4852.5	-47223.9-56928.9	0.180	0.99
BNP M3-M1	-67.81	-151.67-16.05	0.098	-116.29	-227.54-(-5.05)	0.061	0.631
hs-CRP M2-M1	-2.00	-4.74-0.74	0.205	1.39	-9.36-5.74	0.637	0.281
hs-CRP M3-M1	1.00	-5.69-7.69	0.236	-1.81	-9.36-5.74	0.568	0.832

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}).
TNF-α – plasmatic tumor necrotic factor alpha (pg/ml); IL-6 – plasmatic interleukin 6 (pg/ml); sFasL – plasmatic soluble FasL (ng/ml); sCD40 – soluble citokyne 40 (pg/ml); BNP – plasmatic brain natriuretic peptide (pg/ml);hs-CRP – plasmatic C reactive protein (mg/L); EXTG – Exercise training group; CG – Control group

7.1.4. Safety results of Exercise HIIT protocol

During high intensity interval training program there were no complications, namely cardiac, muscular, osteoarticular or other in any of the patients of our population of CRT-advanced heart failure patients.

Only one patient had excessive increase of BP with exercise, which disappeared after adjustment of hypertension medication.

7.1.5. Cardiac Events at 7 months after CRT (M3)

Cardiac events at M3 are reported in this sub-chapter in randomized groups, EXTG and CG. Also, the events in total 121 CRT patients and NEXTG are included here, for easier comparison.

Cardiac events at M3, 7 months after CRT, corresponding to 6 months after exercise program, occurred in 9% of EXTG and 10.7% of CG and were the following, as observed in table 10:

Table 10. Cardiac Events at M3, in total CRT, EXTG, CG and NEXTG

Cardiac Events	Total group		NEXTG		EXTG		CG	
	n	%	n	%	n	%	n	%
Death	6	4.9	6	6	0	0	1	3.5
HF hospital admission	7	5.7	7	7	0	0	1	3.5
Cardiac death/ HF hospital admission	11	9	11	11.1	0	0	1	3.5
Death/hospital admission	14	11.5	13	13.1	1	4.5	3	10.7
Ventricular tachycardia	11	9	9	9	2	9	2	7.1
Death/adm./arrhythmia	18	14.8	16	16.2	2	9	3	10.7

HF hospital admission – hospital admission for heart failure; adm. – admission; NEXTG – Nonexercise group; EXTG – Exercise group; CG – Control group; M3 – moment 7 months after CRT

7.2. Effects of CRT with/without Exercise: An observational study

7.2.1. CRT Cohort sample

From the initial 121 CRT patients, 59 were not randomized because of living distantly from the hospital or reffusal to participate in the EXT trial. These, together with the 12 patients who reffused to exercise after randomization, but accepted the proposed evaluation (71 patients in total), took part of a cohort study for evaluation of CRT effects in HF. All these patients, together with the randomized 28 patients who did not exercise in the CG (99 patients in total) were denominated non exercise training patients (NEXT) and went through the 3 moments evaluation, exactly as the effectively randomized groups (EXT and CG), as illustrated in figure 3.

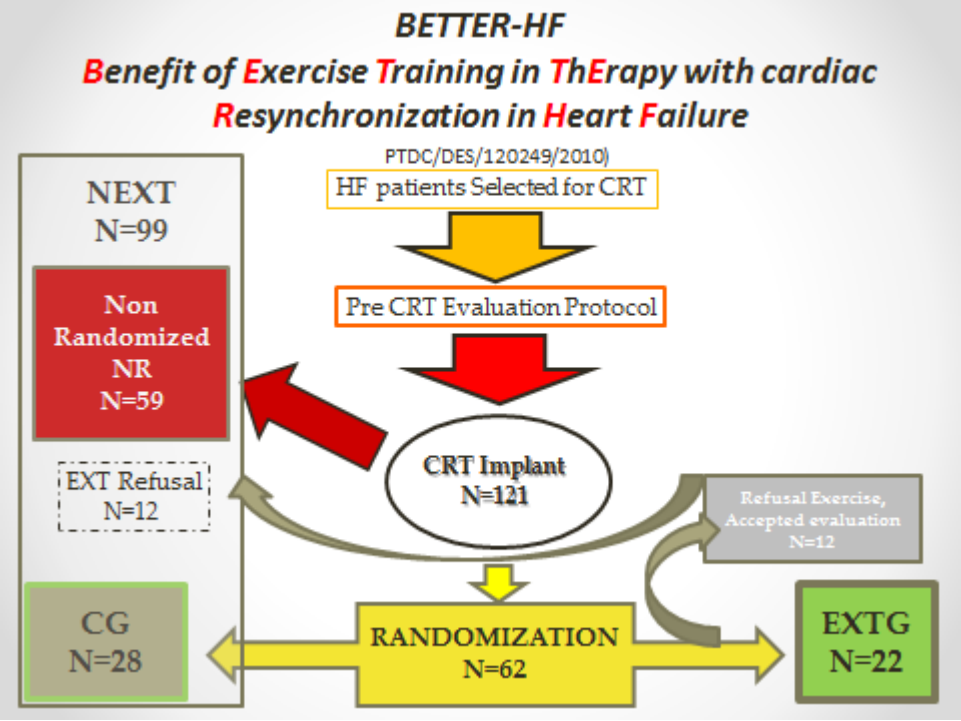

Fig.3. HF-CRT patients (N=121) randomized (n=62) to EXG (N=22) and CG (N=28),
with 12 patients randomized to EXT refusing to exercise but accepting evaluation. Non randomized patients (N=59) accepted evaluation, but performed no EXT.

Demographic and clinical characteristics of the total population sample, including all the patients who did not exercise are included in the next table (table 11).

Table 11. Demographic and clinical characterization of all CRT patients, with and without exercise

	Total	NEXTG	EXTG
N	121	99	22
Age mean±SD (years)	69.6±12.1	69.9±10.8	67.5±9.8
Female (n,%)	38 (31.4%)	33 (33%)	5 (22.7%)
BMI mean±SD (Kg/m2)	26.9±4.8	26.1+3.6	27.18±3
NYHA II-III (n,%)	115 (95%)	94 (94.9%)	21 (95%)
NYHA IV (n,%)	6 (5%)	5 (5%)	1 (5%)
LVEF mean±SD (%)	26.2±7.0	26.1±7.2	26.68±6.21
LVEF<20% (n,%)	27 (22%)	24 (24%)	3 (13.6%)
AF (n,%)	40 (33)	31 (31%)	9 (40.9%)
Ischemic DCM (n,%)	37 (30.5%)	28 (28%)	9 (40.9%)
wQRS mean±SD (msec)	144.3±22.6	141.8±22.2	157.5±21.2

BMI – Body Mass Index (Kg/m^2); NYHA – New York Heart Association clinical functional classification LVEF – Left Ventricular Ejection Fraction (%); AF – Atrial Fibrillation; DCM – Dilated Cardiomyopathy;Wqrs – length of QRS

The results of CRT effects of the total population sample studied as a cohort, without randomization, at M1, M2 and M3 are described in this chapter.

7.2.2. Baseline Dependent Variables in CRT cohort

Dependent variables baseline values, at M1, in the cohort of CRT patients, are expressed as median and interquartile range 25thpercentile-75thpercentile in table 12.

Table 12- Baseline Dependent variables in Total CRT patients

Variables	Median	P_{25}-P_{75}
NYHA (class)	3	2.7-2.89
HQoL T (score)	0.78	0.816-1.118
HQoL Ph/Ps (score)	0.50/1.25	0.56-0.83/1.47-1.53
LVEF (%)	27	24.9-27.44
LVEDVol (ml)	195	193.97-220.43
LVESVol (ml)	144	142.81-164.75
LVMass (gr)	310.66	310.27-345.11
GLS (%)	-7	-7.03-(-5.64)
E/E'	16	15.89-20.88
LAVol (ml)	79	74.99-97.83
RAVol (ml)	36	34.19-52.81
TAPSE (mm)	18	17.89-20.13
PSAP (mm Hg)	38.5	38.08-43.38
HRbas (bpm)	76.5	75.8-82.22
HRmax (bpm)	115	115.52-125.19
SBPbas (bpm)	120	113.91-120.67
SBPmax (mm Hg)	143	135.4-178.17
HRR1 (bpm)	13	10-16
HRR6 (bpm)	35	30-39
CPTdur (sec)	360	315.96-410.84
VATtime (sec)	240	235.83-305.01
VO_{2p} (ml/kg/min)	13	13.27-15.41
$VEVCO_2$ slope	37.9	35.25-40.58
HMRe	1.533	1.444-1.583

HMRl	1.303	1.280-1.408
WOR (%)	48.34	44.65-50.97
RHI	2	1.43-1.68
AI (%)	4	-4.26-5.4
AI@75 (%)	0.01	-5.63-1.73
Wqrs (msec)	150	138.33-153
SDNN	105	108.95-140.07
SDANN	80	76.98-97.62
RMSDD	37	49.33-81.39
pNN50	8	14.87-28.13
BNP (pg/ml)	296.5	395.25-618.22
hs-CRP (mg/L)	2	4.42-10.2
TNF-α (pg/ml)	2	2.22-2.83
IL-6 (pg/ml)	7	5.94-9.62
sCD-40 (pg/ml)	3354	3575.7-5037.37
sFasL (ng/ml)	58	52.79-63.33
NO (µmol/L)	45	0.16-214.7

All values are expressed as median, inter-quartile range 25^{th} percentile-75^{th} percentile (P_{25}-P_{75}). NYHA - New York Heart Association functional clinical classification; HQoL T- total score of quality of life questionnaire HeartQoL; HQoL Ph – Physical score of quality of life questionnaire HeartQoL; HQoL Ps – psychologic score of quality of life HeartQoL ; LVEF – left ventricular ejection fraction; LVEDVol – left ventricular end-diastolic volume; LVESVol – left ventricular end-systolic volume; LVMass – left ventricular mass; GLS – left ventricular Global Longitudinal Strain; E/e'- ratio between E wave from pulsed Doppler left inflow wave ventricular and tissue Doppler mitral annular and mean e'; LAVol – left atrial volume; RAVol – right atrial volume; TAPSE – tricuspide annular plane systolic excursion; PSAP – pulmonary systolic arterial oulmonary pressure; HRbas – baseline heart rate; HRmax –maximal heart rate; SBPbas – baseline systolic pressure; SBPmax – maximal systolic pressure; HRR1 – heart rate recovery at 1^{st} min; HHR6 – heart rate recovery at 6^{th} min; CPTdur – duration of cardiopulmonary testing; VATtime – time to ventilatory anaerobic threshold; VO_{2P} – ventilatory oxygen in consumption; $VEVCO_2$ slope – slope of ventilatory exchange of carbon dioxide; HMRe – ^{123}I-MIBG heart to mediastinum ratio;WOR - wash-out ratio; RHI – reactive hyperemia index; AT – augmentation index; AT@75 – augmentation index corrected for average heart rate of 75 bpm; Wqrs – QRS length; SDNN – standard deviation of NN interval; SDANN – standard deviation of the average N-N interval over periods of about 5 minutes; RMSDD - square root of the mean squared differences between adjacent N-N intervals, NN50 – number of adjacent N-N intervals that differ more than 50 ms;BNP– plasmatic brain natriuretic peptide;hsCRP –plasmatic C reactive protein; TNF-α – plasmatic tumor necrotic factor alpha; IL-6 – plasmatic interleukin 6; sFasL - plasmatic soluble FasL; sCD-40 – soluble citokyne 40; NO – plasmatic nitric oxide

7.2.3. Variation of Dependent variables after CRT in HF patients

7.2.3.1. Clinical functional class and quality of life effects of CRT

Improvement in NYHA functional class occurred in 75.6 % of the total CRT sample patients (clinical responders) at M3, being the variation of clinical functional NYHA class statistically significant at M2 and M3 ($p<0.0001$), for total, NEXTG and, as already observed in the RCT, in EXTG patients, as seen in table 13.

In total CRT sample there were no differences in the rate of response regarding gender, age, etiology and LV dysfunction severity.

In total CRT sample, 80 % of the patients improved Heart quality score after CRT.

Quality of life scores after CRT, total, psychological and physical, assessed by HeartQol questionnaire improved very significantly ($p<0.0001$),as reported in table 14.

There was no difference in gender regarding the effect of CRT on quality of life.

Table 13. NYHA functional class variation after CRT in total group, EXTG and NEXTG

	Total			NEXTG			EXTG		
NYHA Δ	**Median**	**P_{25}-P_{75}**	**P**	**Median**	**P_{25}-P_{75}**	**P**	**Median**	**P_{25}-P_{75}**	**P**
NYHA M2-M1	-0.92	-1.08-(-0.76)	0.000	-0.84	-1.01-(-0.67)	0.000	-1.27	-1.611-(-0.945)	0.000
NYHA M3-M1	-1.01	-1.19-(-0.82)	0.000	-0.88	-1.1- (-0.67)	0.000	-1.52	-1.821-(-1.231)	0.000

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}).
NYHA – New York Heart Association clinical functional classification; M2-M1 – variation from 4 months after CRT to baseline; M3-M1 – variation between 7 months to baseline; NEXTG- Nonexercise Training group;EXTG – Exercise Training group

Table 14. Heart Quality of Life variation after CRT in total group, EXTG and NEXTG

	Total			NEXTG			EXTG		
	Median	**P_{25}-P_{75}**	**P**	**Median**	**P_{25}-P_{75}**	**P**	**Median**	**P_{25}-P_{75}**	**P**
HQol T M3-M1	0.82	0.42-1.1	0.000	0.75	0.51-.99	0.000	1.26	0.84-1.68	0.000
HQol Ps M3-M1	1.1	0.84-1.36	0.000	1.02	0.51-.99	0.000	1.05	0.71-1.39	0.000
HQolPh M3-M1	0.72	0.48-.96	0.000	0.7	0.44-1.7	0.000	1.52	1.23-1.82	0.000

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}).
HQoL T – total score of quality of life questionnaire HeartQol (Oldridge); HQol Ph – physical score of quality of life questionnaire HeartQol; HQol Ps – psychologic score of quality of life questionnaire HeartQol; NEXTG – Non exercise group; EXTG – exercise training group

7.2.3.2. Echocardiographic effects of CRT

After 4 months post-CRT, at M3, patients had a mean LVEF increase of 9.1% and at 7 months a higher increase of 10.7%, being this improvement significant (p<0.0001). The significant increase of LVEF was also maintained when patients submitted to exercise intervention were excluded.

At 7 months, 63.9.% were echocardiographic CRT responders (LVEF variation> 5%). Patients with CRT echocardiographic or clinical response were 100 (82.6%).

LVED volume decreased at 7 months, however not significantly and LVES volume decreased significantly only at 7 months: 22.4 ml mean value reduction (p=0.001).

The variation of ESLV volume attained significance at 7 months, independently of exercise.

LV mass decreased at 4 months, mean value 26.5 g, showing already a tendency for statistical significance (p=0.077), and moreoever at 7 months, LV mass decreased more, 42.1 g, mean value, attaining statistical significance (p=0.025). Excluding the exercise group, the LV mass variation showed a tendency to significance at 6 months (p=0.054).

GLS increased significantly (-1.709±3.871), but only at 7 months and, excluding the exercise group, the statistical significance was maintained.

E/e' changed significantly (E/e' ratio decreased, mean variation 3.334; p=0.039), only at 7 months.

LA volume decreased significantly at 4 months and maintained the decrease at 7 months (mean variation 20.25 ml; p=0.010). RA volume decreased, but nonsignificantly.

TAPSE increased almost significantly at 4 months (p=0.051) although changed not significantly at 7 months. Systolic pulmonary arterial pressure changed non significantly, although with greater decrease at 7 months. Patients who had no criteria for randomization to exercise and did not perform exercise, also showed a significant increase of LVEF, at 4 and 7 months, and a reduction of LVES volume at 7 months. All the described echocardiographic effects are shown in table 15.

Table 15. Echo variables variation after CRT, total group, EXTG, NEXTG

Echo Δ	Total CRT			NEXTG			EXTG		
	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	P
LVEF M2-M1	9.19	7.01-11.36	0.000	8.65	6.49-10.81	0.000	11.90	5.29- 18.50	0.004
LVEF M3-M1	11.83	9.60-14.07	0.000	12.2	9.7-14.7	0.000	11.86	6.66- 17.06	0.001
LVEDVol M2-M1	-7.73	-36.7-21.3	0.875	-7.49	-23.05-8.06	0.166	-8.79	-36.99-19.42	0.594
LVEDVol M3-M1	-15.3	-38.3-8.3	0.279	-13.66	-24.04-(-3.28)	0.011	-21.71	-42.38-(-1.05)	0.019
LVESVol M2-M1	-17.88	-43.08-7.32	0.058	-16.33	-30.06-(-2.6)	0.009	-24	-53- 5.00	0.140
LVESVol M3-M1	-25.13	-45.68-4.5	0.001	-25.91	-35.5-(-16.32)	0.000	-22.24	-38.80-(-0.68)	0.015
LV Mass M2-M1	-26.54	-56.13-3.03	0.077	-34.11	-77.5-10.4	0.645	-7.37	-74.68- 59.93	0.972
LV Mass M3-M1	-22.85	-40.12-(-3.04)	0.026	-18.62	-39.85-2.61	0.079	-38.58	-82.63- 5.48	0.094
LV GLS M2-M1	-1.041	-1.77-0.95	0.004	-1.10	-2.08-(-0.12)	0.039	-0.75	-4.15- 2.65	0.528
LV GLS M3-M1	-1.710	-2.68-(-0.73)	0.003	-1.5	-2.53-.46	0.013	-2.36	-5.53- 0.81	0.109
LV E/e' M2-M1	-1.259	-4.15-1.63	0.008	-1.29	-4.79-2.22	0.391	-0.88	-5.42- 3.67	0.599
LV E/e' M3-M1	-3.145	-6.48-(-0.18)	0.009	1.53	1.50-4.55	0.069	-6.4	-12.28-(-0.52)	0.042
LAVol M2-M1	-16.900	-30.15-(-3.64)	0.025	-18.81	-32.84-(-4.78)	0.008	3.33	-29.02- 35.69	0.917
LAVol M3-M1	-6.883	-17.46-3.69	0.09	-6.44	-18.61-5.72	0.137	-9.14	-33.86- 15.58	0.499
RAVol M2-M1	-7.26	-14.65-0.11	0.054	-5.85	-13.41-1.71	0.091	-15.67	-37.94- 6.60	0.074
RAVol M3-M1	-3.43	-10.47-3.59	0.329	-2.23	-9.93-5.48	0.262	-11.71	-30.43- 7.00	0.150
TAPSE M2-M1	1.28	-0.05-2.58	0.051	-0.84	-.54-2.22	0.170	1.67	-1.41-4.75	0.167
TAPSE M3-M1	-0.52	-1.86-0.80	0.430	-0.85	-2.26-.57	0.353	0.5	-2.42-3.42	0.893
PSAP M2-M1	-1.8	-5.36-2.40	0.448	-1.07	-5.01-2.88	0.668	-2	-13.75- 9.75	0.858
PSAP M3-M1	-2.39	-6.66-1.89	0.103	-2.41	-7.11-2.23	0.397	-2.29	-16.11-11.54	0.400

¶

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}).
LVEF – left ventricular ejection fraction (%);LVEDVol – left ventricular end-diastolic volume (ml);LVESVol – left ventricular end-systolic volume (ml); LVMass – left ventricular mass (g); LV GLS – left ventricular Global Longitudinal Strain (%); LV E/e'- ratio between E wave from pulsed Doppler left ventricular inflow wave and tissue Doppler mitral annular and mean e'; LAVol – left atrial volume (ml); RAVol – right atrial volume (ml); TAPSE – Tricuspide Annular Plane Systolic Excursion (mm); PSAP- pulmonary systolic artery pressure (mm Hg); NEXTG - Non exercise training group; EXTG – Exercise Training group

7.2.3.3. Exercise functional testing effects of CRT

Baseline and maximal heart rate did not change significantly.

The variation of HR at 1st minute recovery and at 6th min recovery regarding the peak HR (reduction) was significant, at 7 months after CRT.

There was a significant variation of VO_{2p}, at 3+1 months (mean increase of 1.79 ml/kg/min), maintaining the functional benefit at 6 months.

Most of our patients increased VO_{2p}. 76 (62.8 %) patients were functional responders (variation>1ml/kg/min).

CPT duration increased significantly at M2 and M3 (p=0.000 and p=0.002, respectively) and time to AT increased significantly at M3 after CRT (p=0.001).

VE/VCO2 slope, decreased significantly at 3 and 6 months (p=0.000 and p=0.003, respectively).

Exercise testing variables are shown in table 16.

Table 16. Exercise Testing variables variation after CRT: total sample, NEXTG, EXTG

CPT Δ	Total CRT			NEXTG		P	EXTG		
	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}		Median	P_{25}-P_{75}	p
HR bas M2-M1	-2.22	-5.97- 1.52	0.240	-3.65	-7.78-0.49	0.136	1.56	-7.07- 10.19	0.469
HR bas M3-M1	-3.72	-7.79-.35	0.073	-3.56	-8.1-0.98	0.156	-4.47	-12.95- 4.01	0.443
HR max M2-M1	0.94	-3.90-5.79	0.699	0.88	-4.33-6.09	0.341	0.88	-11.66-13.41	0.552
HR max M3-M1	-2.12	-7.51-3.26	0.435	-2.33	-8.24-3.57	0.699	-3.59	-16.72- 9.54	0.795
HRR1 M2-M1	-0.88	-6.06-4.29	0.734	-2.8	-8.8-3.19	0.368	6.5	-2.62- 15.62	0.079
HRR1 M3-M1	-6.65	-11.94-(-1.35)	0.015	-7.89	-13.91--1.88	0.024	-2.19	-12.73- 8.35	0.979
HRR6 M2-M1	-3.13	-6.73-0.46;	0.087	-4.24	-8.44-(0-.04)	0.082	2.46	-3.67-8.60	0.25
HRR6 M3-M1	-4.04	-7.75-(-0.33)	0.033	-4.7	-8.75-(-0.64)	0.020	-1.76	-9.76-6.22	0.76
SBP bas M2-M1	7.27	1.83-12.71	0.007	6.36	-0.19-12.91	0.045	10.56	1.10-20.02	0.033
SBP bas M3-M1	4.81	0.89-8.72	0.020	4.35	-0.09-8.79	0.071	6.33	-2.82- 15.49	0.141
SBPmax M2-M1	13.27	5.82-20.73	0.001	13.4	4.62-22.19	0.004	12.81	-2.32- 27.95	0.093
SBPmax M3-M1	7.61	1.59-13.63	0.004	6.03	-1.25-13.32	0.038	12.78	2.36-23.20	0.034
CPTdur M2-M1	85.52	39.57-131.48	0.000	62.29	-16.49-108.09	0.024	167.69	38.9-296.47	0.017
CPTdur M3-M1	80.08	32.89-137.71	0.002	37.31	-7.12-81.74	0.11	235.13	83.07-387,18	0.008
VATtime M2-M1	31.23	-19.47-81.95	0.271	-34.5	-77.65-8.65	0.156	174.64	86.88- 262.39	0.006
VATtime M3-M1	90.5	39.5-141.64	0.001	61.54	-6.71-129.79	0.085	216.42	109.7-323.14	0.004
VO2p M2-M1	1.7	0.203-3.209	0.027	1.6	0.17-3.03	0.041	2.44	-0.05;4.92	0.026
VO2p M3-M1	1.45	- 0.06-2.85	0.040	0.6	0.78-1.99	0.381	2.18	-0.53- 4.80	0.08
Ve/VCO_2sl M2-M1	-6.8	-9.50-(-4.02)	0.000	-7.53	-10.53-(-4.52)	0.000	-3.62	-9.64- 2.41	0.294
Ve/VCO_2sl M3-M1	5.31	-8.77-(-1.858)	0.003	-6.67	-10.76-(-2.57)	0.005	0.08	-4.88-5.03	0.551

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}).
HRbas – baseline heart rate (bpm); HRmax – maximal heart rate (bpm); SBPbas – baseline systolic pressure (mm Hg); SBPmax maximal systolic pressure (mm Hg); HRR1- heart rate recovery at 1st min; HHR6 – heart rate recovery at 6th min (bpm), CPTdur – duration of cardiopulmonary testing (sec), VATtime – time to ventilatory anaerobic threshold (sec), VO_{2P} – ventilatory oxygen consumption (ml/kg/min); $VEVCO_2$ slope – ventilatory minute – exchange of carbon dioxide relation slope; NEXTG – Nonexercise training group; EXTG- exercise training group

7.2.3.4. Imaging autonomic system function effects of CRT

In our total HF patients sample, as a whole, we found nonsignificant variation of ^{123}I-MIBG cardiac scintigraphy parameters, HMRe, HMRl and WOR, after CRT (table 17).

Evaluating separately the nonischemic cardiomyopathy patients, we found that HMR late and WOR, as continuous variables, correlated significantly with CRT echocardiographic response, by univariate analysis, which did not occur in ischemic HF patients. In this nonischemic group of CRT patients, those with baseline HMRl >1.5 had a 3 fold greater possibility of being an echocardiographic responder (LVEF>5%), compared to those with HMRl<1.5. This association was confirmed by multivariate analysis (p=0.05).

Table 17. MIBG$_{123}$-Cardiac Scintigraphy variables variation after CRT: total sample, NEXTG, EXTG

MIBG scint. Δ	Total CRT			NEXTG			EXTG		P
	Median	P_{25}-P_{75}	p	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	
HMRe M3-M1	-0.017	-0.578-0.021	0.37	-0.012	-0.14-0.1	0.75	-0.04	-0.42-0 .21	0.508
HMRl M3-M1	-0.039	-0.893-0.009	0.115	-0.07	-0.19-0.05	0.411	-0.02	-0.3- 0.23	0.937
WOR M3-M1	1.901	-4.849-8.651	0.574	3.56	-2.46-9.59	0.361	-3.34	-48.13- 34.16	0.642

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}).
HMRe – ^{123}I-MIBG early heart to mediastinum ratio; ^{123}I-MIBG HMRl – late heart to mediastinum; ^{123}IMIBG WOR-wash-out rate; NEXTG – Non Exercise Training Group; EXTG – Exercise Training group

Endothelial effects of CRT

The studied parameters, for endothelial function, RHI, AI and AI@75 did not vary significantly after CRT in the whole CRT population sample. NO also did not vary significantly, neither was associated with CRT response. All parameters increased, although nonsignificantly, as seen in the next table (table 18).

Table 18. Peripheral Artery Tonometry effects of CRT

Endopat and NOΔ	Total CRT			NEXTG			EXTG		
	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	P
RHI M3-M1	0.88	0.078-0.44	0.659	1.1	-1.22-3.34	0.705	0.20	-0.17-0.57	0.257
AI M3-M1	9.37	2.46-21.21	0.110	1.89	-7.65-11.44	0.997	8.94	-2.16- 20.04	0.107
AI@75 M3-M1	8.31	2.81-19.43	0.130	1.34	-5.65-8.33	0.920	8.24	-2.16- 18.63	0.107
NO M3-M1	-87.82	-257.17-81.53	0.240	-108.83	-316.18-98.52	0.286	7.66	-27.71-43.03	0.657

All values are expressed as median, inter-quartile range 25^{th} percentile-75^{th} percentile (P_{25}-P_{75}).
Reactive Hyperemia Index; AI – Augmentation Index (%); AI@75 – Augmentation Index adjusted for average heart rate of 75 bpm (%). NO – Nitric oxide (μmol/L); NEXTG – Nonexercise training group; EXTG – Exercise training group

7.2.3.5. Inflammation and Apoptosis biomarkers variables variation after CRT

In the total HF patients, BNP decreased nonsignificantly, as hs-CPR, which only decreased at M2.

Inflammatory parameters, sCD-40 and sFasL, increased after CRT, but only significantly at M2 for sCD-40. IL-6 had also nonsignificant variation. TNF-α decreased at M2 and especially at M3 after CRT, but nonsignificantly.

Biomarkers data are included in the next table (table 19).

Table 19. Biomarkers variation after CRT: total sample, NEXTG, EXTG

Inflammatory Markers Δ	Total CRT			NEXTG			EXTG		
	Median	P_{25}-P_{75}	p	Median	P_{25}-P_{75}	P	Median	P_{25}-P_{75}	p
TNF-α M2-M1	- 0.078	-0.759-0.602	0.81	-	-0.87-0.44	0.647	0.18	-1.92; 2.28	0.439
TNF-α M3-M1	-0.108	-408-0.190	0.47	0.11	-0.20-0.41	0.836	-1.08	-1.84; -0.32	0.016
IL-6 M2-M1	1.245	-1.897-4.388	0.39	1.44	-2.56-5.45	0.374	0.5	-5.85; 6.85	0.317
IL-6 M3-M1	-0.730	-3.297-1.837	0.56	-1.01	-3.96-1.93	0.495	-0.33	-7.65; 6.99	0.684
SFasL M2-M1	11.360	-1.6-24.37	0.083	13.56	-4.24-31.35	0.074	-	-	-
SFasL M3-M1	4.113	-0.79- 9.02	0.098	3.82	-1.11-8.76	0.224	5.29	-14.71; 25.28	0.310
sCD40 M2-M1	5897.16	-1436.411-10357.921	0.01	8041.17	5279.22-10803.11	0.109	-	-	-
SCD40 M3-M1	2090.88	-1563.806-5745.573	0.23	2090.88	-1563.81-5745.57	0.209	3753	-9885; 17391	0.180
BNP M3-M1	-50.742	-178.13-42.64	0.22	-42.72	-5.86-6.79	0.153	-67.81	-151.67; 16.05	0.098
hs-CRP M2-M1	-0.044	-5.05-4.96	0.98	0.46	-2.11-6.45	0.469	-2.00	-4.74; .74	0.205
hs-CPR M3-M1	1.933	-1.69-5.55	0.29	2.17	-2.11-6.45	0.484	1.00	-5.69; 7.69	0.236

TNF-α – plasmatic tumor necrotic factor alpha (pg/ml); IL-6 – plasmatic interleukin 6 (pg/ml); sFasL – plasmatic soluble FasL (ng/ml); sCD-40 – soluble citokyne 40 (pg/ml); BNP – brain natriuretic peptide (pg/ml); hs-CRP – plasmatic C reactive protein (mg/L); NEXTG – Non Exercise Training group; Exercise Training group

7.2.4. EXTG and NEXTG: Comparative effects

Comparing EXTG with NEXTG variation of dependent values, there was a statistical difference observed at M3, only in the variables, NYHA, CPET, AT time, TNF@, as shown in table 20.

Table 20. Comparison of Effects in EXTG and NEXTG with statistical significance

	EXTG		NEXTG		
	Median	P_{25}-P_{75}	Median	P_{25}-P_{75}	P
NYHA class M3-M1	-1.52	-1.84-(-1.23)	-0.88	-1.1-(-0.67)	0.004
CPT dur M3-M1(msec)	235.13	83.07-387.18	37.31	-7.12-81.74	0.001
ATtime M3-M1(msec)	174.64	109.7-323.14	-34.5	-6.71-129.79	0.000
TNF@ M1-M3 (pg/ml)	1.08	-1.84-(-0.32)	0.11	-0.20-0.41	0.01

All values are expressed as median, inter-quartile range 25th percentile-75th percentile (P_{25}-P_{75}).
New York Heart Association clinical classification; CPTdur – cardiopulmonary exercise testing duration; ATtime – time to anaerobic threshold; TNF-α – Tumor Necrotic Factor alpha; EXTG – Exercise group; NEXTG – No exercise group

7.2.5. Effects in responders and non responders to CRT, with and without Exercise

Patients in this study were mostly in class III/IV, NYHA (74.3 %) and were considered to respond to CRT at M3, clinically (≥1 class NYHA) in 91 cases (75.6%), echocardiographically (LVEF variation greater than 5% increase) in 77 cases (63.9%) and functionally (VO_{2p} variation greater than 1ml/kg/min) in 76 (62.8 %) patients.

Patients with both clinical and echocardiographic response were 59 (48.7%), while those with either clinical or echocardiographic response were 100 (82.6%).

Comparative effects of CRT in echocardiographic responders and nonresponders, with significant difference of all CRT patients of our study are shown in table 21.

Table 21. CRT Responders vs Non responders: Baseline and Effects difference

	CRT responder	CRT non responder	P
Baseline M1			
SDNN	134.843±75.72	103.440±43.04	0.024
HMRe	1.525±.16	1.477+.14	0.034
HMRl	1.432±.18	1.360±.15	0.028
LVED	196.92±72.69	228.34±67.62	0.032
LVES	154.46±59.72	162.07±57.32	0.000
TAPSE	20.077±5.346	17.229±5.618	0.016
Variation M3-M1			
ΔLVEF	18.14±9,61	-10.39±12,80	0.000
ΔLVESVol	-37.186±40.148	4.206±38.805	0.000
ΔGLS	-2.564±3.757	-0.247±3.218	0.002
ΔVO2p	2.795±5.15	0.488±3.86	0.058

All values are expressed as mean±SD. SDNN- standard devistion of NN interval, HMRe- Early heart-mediastinum ratio; HMRl- Late heart-mediastinum ratio; LVEDVol – left ventricular end-diastolic volume (ml);LVESVol – left ventricular end-systolic volume (ml); TAPSE – Tricuspide Annular Plane Systolic Excursion (mm); ΔLVEF – variation of left ventricular ejection fraction; ΔLVESVol – variation of end-systolic left ventricular volume; ΔGLS- variation of global longitudinal strain; ΔVO2p-variation of peak oxygen consumption; NEXTG - Non exercise training group; EXTG – Exercise Training group

Responders had less events: carardiac death+HF hospital admissions; death+hospital admissions+arrhythmias.

Responders, with non ischemic HF, also had statistically more frequent baseline HMRl>1.5: OR 1.8 (95%CI 1,11-2,99).

For evaluation of predictive factors to CRT response, In a multivariate analysis which included clinical, functional and echocardiographic variables, TAPSE was found to be the only variable associated to response, predicting higher values a positive response to CRT (OR=1.13;95%CI:1.02-1.26; P=0.020). TAPSE<15 was associated to nonresponse to CRT (p=0.05) and there were no responders when baseline TAPSE <10 (submitted paper).

8. DISCUSSION

The discussion of results will be done separately regarding the exercise RCT, BETTER-HF, which is the purpose of this thesis (chapter 8.1), and the CRT cohort, relevant for a deeper understanding (chapter 8.2).

8.1. High intensity Interval Training after CRT: A randomized control trial

Effects and mechanisms of Exercise training in HF-CRT patients

The purpose of evaluating a specific protocol of high intensity interval training in an optimized pharmacological and CRT treated patients with advanced heart failure is ambitious. We are not really addressing the issue of "exercise effects in HF", but of "exercise larger additional effects after CRT", regarding those already well demonstrated pharmacologic and CRT effects in large HF trials.

Clinical functional class and quality of life EXT effects in HF-CRT patients

Overt heart failure is manifested by decreased effort tolerance and increasing disability, with daily life severe restrictions. As we are fully aware, despite being subjective, the clinical functional classification of New York Heart Association (NYHA), is the most frequently used method to quantify clinical severity of heart failure[490].

The positive effect of a therapeutic intervention on symptoms severity and decreased quality of life, obviously important from the patient's own perspective, is though to be fundamental for HF patient's wellbeing.

In our randomized groups, both submitted to CRT, clinical functional class improved in both exercise and control groups, more significantly in the exercise group, after 6 months of exercise training (p=0.034).

Clinical response, defined by the decrease of 1 clinical functional NYHA class, was present in 90.1%, at 3 months and in 95%, at 6 months of EXT (M3) in the EXTG versus 82.6% and 78.5%, respectively, at the same moment in the CG. It is interesting to notice

that, although at 6 months the percentage of clinical nonresponders was more than 4-fold greater in CG, 21.5% (compared to 5% in EXTG), this difference did not reach statistical significance, most probably due to the fact of the small number of responders and nonresponders in the two groups, analysed in this sample. Despite this fact, these results, in our patients, point out to a relation of HIIT intervention with decreased number of clinical nonresponders, therefore suggesting an additional effect of exercise in the improvement of symptoms clinical severity, with benefit for a greater number of patients (16.5% less of nonresponders).

Besides this more subjective clinical functional class improvement evaluation, the quality of life (QOL), objectively quantified by a score derived from HeartQol questionnaire[491], also improved very significantly (p=0.000), in all dimensions (total, psychological and physical), in both randomized groups, with and without exercise, with no statistical significant difference. 95% in EXTG and 96% in CG had an improvement of HQoL considered at least moderate. It is interesting to notice that all randomized patients, except one, improved the quality of life scores, and that the one who did not increase belonged to the control group (3.5% non improvement of QOL for CG vs 0% for EXTG).

Other authors have also shown before, the benefit in quality of life of CRT or exercise, using different instruments[20].

It is easily understandable that patients who were previously very disabled, reporting clinical functional improvement after intervention, might undoubtly gain the frequently lost quality of life. Although QOL is probably the most important endpoint from a patient's point of view and is quantitatively translated into a score, which is considered objective, we must not forget that QOL measurement is also subjected to some bias and to personal interpretation.

Echocardiographic EXT effects and mechanisms in HF-CRT patients

An important issue to be discussed is the effect of exercise after CRT on cardiac remodeling.

Preventing or reversing maladaptive remodeling is an important therapeutic target, since cardiac remodeling is a central factor of disease progression in HF patients[324-325].

The relation between exercise training and cardiac remodeling has been already described. Looking back to the first larger prospective randomized study, conducted by Hambrecht *et al.*[459], which provided evidence for training-induced reverse remodeling, it has been demonstrated that endurance training led to reverse left ventricular (LV) remodeling, although with modest improvements in LVEF (from 30% to 35%), as well as LV end-diastolic diameter reduction.

These results were confirmed by two meta-analyses[460,461], despite some of the included studies[501,502] not having positive effect on LV volumes reduction. These meta-analyses also demonstrated that aerobic training, especially greater than 6 months duration, significantly reversed LV remodeling, whereas strength training, alone or combined with aerobic training, had no effect on reverse remodeling. This conclusion might have been influenced by the different number of patients analysed, with a smaller number in isolated or associated strength training, as already discussed. Still, there were doubts in these studies, regarding the central effect of exercise.

Wisloff *et al.* performed the study[445], which inspired several other investigators, demonstrating in a small sample of patients that high-intensity interval training relative to the individual's maximal oxygen uptake is feasible, even in elderly patients with chronic heart failure and severely impaired cardiovascular function. These authors confirmed that the intensity of exercise may be an important factor for reversing LV remodeling. The underlying physiologic concept behind interval training regards the fact that metabolic rate is raised for a brief period, considered higher than for a typical continuous exercise program, which allows a longer duration of a given training period to be spent at a higher percentage of peak oxygen consumption (VO_{2p}). This has the effect of eliciting a higher rate of energy production, requiring different metabolic pathways to produce energy and different muscle fiber recruitment patterns from those elicited by continuous training[503].

Facing all the previous data, suggesting greater cardiovascular adaptations after high-intensity interval exercise, we decided in our study, to use HIIT, with a long duration exercise program, for 6 months, to allow time for remodeling, which might take longer than 3 months, with a frequency of twice a week and duration of 60 minutes per session. The inclusion of a third session per week might had been important, nevertheless the

acceptance and adherence of patients for this long period of 6 months would probably had been worst.

More recent studies, using HIIT exercise, have suggested or demonstrated that cardiac function can also be improved[504].

In our study, LVEF, at M2 (3 months of exercise), increased significantly in patients from both randomized groups, with greater magnitude (although nonsignificantly different) in those from exercise group (LVEF mean increase of 11.9% in EXTG vs 9.6% in CG). At 6 months after exercise, at M3, the improvement continued to be greater, but with a smaller difference (11.9% for EXTG vs 10.4% for CG; p=ns). Eventhough the LVEF difference was small at 6 months, we have to keep in mind that these patients have baseline severe LV dysfunction, with a mean LVEF of 26%, which is increased by CRT, as demonstrated in multiple studies. Also, apparently small LVEF improvement differences might represent a valuable percentual gain (mean LVEF relative increase of 46% in the EXTG and 40% in the CG with a mean difference of 6%). The worst the baseline LVEF it is, the more important is getting a greater LVEF increase after HIIT. What we have noticed in this sample, was that patients undergoing HIIT attained faster a better LVEF and maintained a slightly higher LVEF increase, which might be clinically important, especially because of the known prognostic value of LVEF[505].

LVEF response was defined as obtaining at least 5% absolute increase of LVEF, which corresponds to 19% and 15% relative increase regarding, respectively, the mean value and the higher value of baseline LVEF in our patients, offering no doubts regarding LV systolic function improvement, largely overpassing the intra and inter-individual variability of LVEF determination by echocardiography[506]. This criterion for echocardiographic response allow us high specificity in CRT response diagnosis, without loosing too much sensitivity.

At M2 (3 months of exercise) and at M3 (6 months of exercise), 68.4 % and 81.8% echocardiographic CRT responders, respectively, were observed in the exercise group and 75 % and 72.7%, respectively, in the CG. The increased number of echocardiographic responders, after 6 months of HIIT program, had no significant statistical difference regarding those who did not exercise, although the number of echocardiographic non responders was 2-fold greater in the control group: 18.2% nonresponders in the EXTG and 27.3% in the CG. The HIIT intervention resulted in a

decrease in the number of echocardiographic non responders, which is particularly important in terms of prognosis for these patients. With a greater sample, in the exercise group, probably the difference might had attained significance.

LV volumes decreased significantly in both groups after 6 months of exercise and at the same time for CG, without statistical difference. Also, only LVES volume decreased significantly at 3 months of exercise, in CG, however without any statistical difference. The LV mass decreased nonsignificantly in EXTG at M1 and continued to decrease, 38.6 g median variation M1-M3, with a tendency to significance (p=0.09), compared to nonsignificant initial increase and posterior small decrease of 11.9 g, in CG, leading to almost 4-fold greater LV mass decrease after 6 months of exercise. We think that if the number of patients would had been greater or if we had continued the exercise after 6 months and performed the echocardiogram later, we might have observed significance in the LV mass improvement in the EXTG. It looks that HIIT may have an additional benefit to CRT on LV remodeling, acting through LV mass reduction, but this tendency needs to be confirmed.

Mechanisms explaining reverse remodeling by exercise training in heart failure have been studied. In the absence of myocardial biopsies, for molecular analysis of myocardial changes induced by training, most investigators interpreted this favorable training effect as secondary to afterload reduction with reduced resting blood pressure due to improved endothelial function[459], as previously discussed.

At cellular level, cellular remodeling is observed in the form of changes in cardiomyocyte size and shape and by molecular modifications that often recapitulate fetal gene expression and compromise excitation–contraction coupling, myofilament function, cell-survival signaling, bioenergetics, and the cellular metabolic state. In addition to the remodeling of cardiomyocytes, remodeling of the extracellular matrix is central to deforming the cardiac chamber and to altering the composition of fibrous and vascular elements in the myocardium. Chamber dilatation typically is also associated with a more-spherical shape of the ventricle, which reduces the efficiency of ejection because cardiomyocytes need to shorten more to achieve the same ejected net volume. Hypertrophy stimulated by stretch (that is, by volume overload), is often termed eccentric and differs from concentric hypertrophy induced by pressure overload[507]. Differences include the shape of cardiomyocytes (long and thin versus short and fat),

the organization of sarcomeres ('in series' versus 'in parallel'), and changes in molecular signaling[508].

Cardiac hypertrophy—irrespective of cardiac morphology—has been traditionally considered to be an adaptive process, at least initially. However, this notion is increasingly questioned. Clinical and experimental data suggest that, if the stimulus for hypertrophy is pathologic, the remodeling response is also pathologic and suppression of remodeling is, therefore, beneficial. Animal studies have shown that even transient exposure to pathological stress induces very different molecular cascades, suggesting that the response to pathological stress is never truly 'adaptive'. Data from trials in humans have confirmed LV hypertrophy as an independent risk factor, and indicate that antihypertensive agents that reduce LV mass lower mortality. Whereas concentric LV hypertrophy often evolves into a more dilated failure phenotype in small rodents, this evolution is less frequently observed in humans. Nonetheless, there is growing sentiment that directly targeting pathological remodeling might be beneficial even if abnormal loading persists. Although therapeutic interference with some signaling pathways can cause adverse effects, accumulating evidence indicates that many maladaptive responses can and should be targeted [509].

In this study, we have not performed myocardial biopsies that could explain reverse remodeling at cellular level, however the significant increase of LVEF, with LV volumes decrease and LV mass decrease (tendency to significance), demonstrated after CRT a positive effect on reverse remodeling, however without significant difference between EXTG and CG.

We also analysed, the effect of exercise on diastolic function. E/e', decreased in both groups, but only significantly in the EXTG, after 6 months of exercise (p=0.04).

Although the reduction of E/e' was 1.5 fold greater in the EXTG, the difference between the 2 randomized groups did not reach statistical significance. We may again argue that in a larger sample or with a longer exercise protocol we might achieve this significance. Further investigation will be needed to understand the additional effect of HIIT to CRT on diastolic function, including other diastolic function parameters.

Regarding all other echocardiographic parameters, no statistical significance was obtained in variation, at 3 or 6 months of exercise, although their variation occurred in the same direction as in the total CRT population. As referred before regarding other

echocardiographic variables, the reason for not having obtained statistical significance in the variation of several echocardiographic parameters after HIIT, relates most probably with the dimension of the analyzed groups. Nevertheless, this is one of the largest studies regarding HIIT in advanced HF patients with CRT, until now.

Exercise functional EXT effects and mechanisms in HF-CRT patients

The functional improvement effect in HF-CRT patients after EXT, using a continuous protocol has been previously demonstrated by objective VO_{2p} increase, by several authors[20,482,483,510].

Also, the benefit of intervalic protocols, HIIT, in functional capacity was demonstrated by Wisloff and others[445]. In these studies, the comparison of VO_{2p} increase between HIIT and MICE was analysed. While some obtained a significant difference with higher benefit for HIIT[445,446,511,512], others did not confirm these results[513,514].

Meyer's meta-analysis[504], including all these studies, confirmed the peripheral functional effect of HIIT, with controversial effect on LVEF, only verified by Wisloff in a sample of 27 patients, inferior to ours, as already mentioned in the discussion of echocardiographic effects.

In our EXT protocol, we included strength training to HIIT, based on the knowledge that associated modalities of training are more effective in improving functional capacity: combined strength and intermittent exercise appear superior for peak VO2 changes when compared to intermittent exercise of similar exercise energy expenditure[515].

Functional benefit was objectively demonstrated in our CRT patients who underwent the HIIT program.

From CPT variables, CPT duration showed a significant increase at 3 months, but especially at 6 months of exercise, in the EXTG, with a significant difference regarding the control group (235 vs 24 sec; p=0.002), whose patients had a nonsignificant decrease in CPT duration.

Also, time to VAT showed significant increase, only in the exercise group, decreasing nonsignificantly in CG, with a significant difference at 3 months (p=0.006) and almost significant at 6 months (216 vs 50 sec; p=0.064), between EXTG and CG.

VO_{2p} increased, though without statistical significance, in all randomized patients, at 6 months, more in the exercise group (EXTG 2.1 ml/kg/min vs CG 0.25ml/kg/min).

We admit that, if the number of our patients was higher, or if we had prolonged the duration of exercise, probably we would also had obtained a significant increase in VO_{2p}. This was the case in the study of Belardinelli[516], in which patients underwent EXT for 10 years , with increasingly VO_{2p}, especially evident until 1 year, but maintaining the difference in the following years regarding those HF patients who did not exercise. Even more, we must keep in mind, the clinical significance of VO_2 improvement, knowing that the increase of 1ml/kg/min associates to 10% decrease of CV mortality[517] and that for each 1% VO_{2p} increase, mortality decreases 2%[518]. According to these data, the VO_2p increase at 6 months in the exercise group can be associated to 20% decrease of CV mortality and more than 2% decrease of total mortality in these patients, reflecting the protective effect of exercise.

Looking at the number of patients who were functional responders (defined as those who increased $VO_2p \geq 1$ml/kg/min), at 3 months, 77.2% in EXTG were responders vs 53.5% in CG, maintaining this rate of response at 6 months. The number of functional nonresponders was less than half in the EXTG, 22.8%, compared to CG, 46.2%, which has clear clinical and prognostic implications.

Despite the VO_2p nonsignificant increase, though superior in our EXTG patients, the significant increase in the duration of exercise testing (CPTdur) and in time to anaerobic threshold (VATtime) attest for a better functional capacity, in these baseline low-functional capacity patients, with beneficial implications for patients life. The positive changes achieved on these exercise testing parameters, attest for the fact that HIIT exercise training elicit a strong functional improvement effect after CRT, which has an important significance to prognosis.

Maximal systolic blood pressure increased more in the EXTG, only significantly after 6 months of exercise (p=0.034), 4-fold more than in control group (variation of 12.8 vs 3 mm Hg), however without statistical difference between the 2 randomized groups. It is important to notice that the absolute mean value of baseline SBP was lower in EXTG, allowing that even with greater SBP variation in these patients, the maximal SBP remained inferior to that of CG patients.

Before analyzing possible autonomic effects, we should remark that baseline HHR1, was not different between the 2 groups and SDNN and other parameters of HRV were in the normal range and also not significantly different between the two groups[519]. Heart rate recovery at first minute (HRR1) was significantly different at 3 months in the 2 randomized groups (p=0.024), with nonsignificant increase in EXTG and nonsignificant decrease in CG. Later, at 6 months, a nonsignificant reduction of HRR1 was observed in the EXTG, compared to a significant decrease in the CG (p=0.039), but no statistical difference between the variation in the 2 randomized groups was demonstrated. Only at 6 months the benefic variation of HHR1 attained significance in the CG, suggesting an effect only of CRT on the ANS. HIIT could not show an additional effect to CRT on this marker of autonomic function, HHR1, which cannot be generalized to no positive effect of exercise on ANS, already demonstrated for EXT in HF, in previous studies[513]. We may hipothesize, looking at our results, that the ANS modulation by HIIT might possibly increase with prolongation of exercise after the 6 months.

Regarding the change of all other CPT variables, no significant difference was shown.

Imaging autonomic system function EXT effects in HF-CRT patients

Advanced HF patients usualy present considerable changes in cardiac autonomic nervous system function.

In our study, patients presented low baseline values of HMRe and HMRl, inferior to normal, indicative of decreased cardiac innervation and inferior to the value 1.6, considered of low prognosis: HMRe 1.51 and HMRl 1.47 in EXTG and HMRe 1.47 and HMRl 1.46 in CG. Baseline WOR values were high, also indicating denervated hearts: WOR 51.34 % in EXTG and WOR 48.24% in CG.

Exercise training can induce, besides haemodynamic and metabolic changes, important neurohormonal adaptations in patients with chronic heart failure due to severe left ventricular dysfunction. Several studies were conducted on the effect of exercise in ANS function, measured by ^{123}I-MIBG cardiac scintigraphy, in HF patients.

Previously, Agostini *et al*. (520) examined the impact of exercise rehabilitation on cardiac neuronal function using ^{123}I-MIBG scintigraphy, in only 14 HF patients (NYHA class II-III), with LVEF<50%, who underwent progressive, supervised continuous

endurance training for 6-month (60 sessions, 3 sessions per week). The authors concluded that exercise rehabilitation induced improvement of cardiac neuronal function without negative effects on cardiac contractility, in patients with stable chronic heart failure.

Contrarily to this study, the comparison of HMRe, HMRl and WOR variation in our patients, in the exercise and control groups, did not show statistical difference. Although without statistical significance, it was interesting to notice that WOR mean value decreased in the EXTG, differently from the increase verified in the CG, which may be prognostically different, once low WOR was associated to poor prognosis, as demonstrated by Kuwabara *et al.*[521], in a nice meta-analysis. This, including 15 published ^{123}I-MIBG studies conducted in Japan, reported that both an increased washout rate and a decreased cardiac ^{123}I-MIBG activity (H/M) were indicative of a poor prognosis in patients with chronic heart failure. According to these data, it is expectable that strategies that reduce WOR should carry an improved prognosis.

It is important to point out that normal WOR values are considered inferior to 10%, but in patients with dilated cardiomyopathies washout rates of ^{123}I-MIBG are typically accelerated between early and delayed images, with values superior to 25% [78]. Some authors demonstrated that values of WOR greater than 27% were associated with a significant increase in sudden death and with a cardiac death rate of 35%[522], while others stated that values of WOR greater than 50% were associated with an increased risk for cardiac death[78,523,524].

In our patients, in the EXTG the baseline WOR median value was 50,5% and after EXT program decreased to 47.16 % (median variation 3.34%), decreasing the risk of cardiac death, according to the above referred studies. Contrarily, in the CG the WOR increased, although nonsignificantly.

The decrease of WOR after exercise, in our patients, despite nonsignificant, may suggest a better prognosis, even more with the presence of high baseline WOR values in these advanced HF-CRT patients. In a larger EXT group or, as already discussed for HRR1, prolonging the duration of EXT, we might had confirmed that EXT after CRT could positively modulate autonomic nervous system, improving cardiac autonomic function.

Endothelial function EXT effects in HF-CRT patients

Besides the myocardium, the vascular system is significantly impaired in HF and several studies using EXT as a therapeutic intervention proved beneficial effects on this system. Reactive hyperemia (RHI), translating endothelial function and arterial index, related to arterial stiffness (not necessarily indicating endothelial function) were measured in our study.

On a functional level, it has been shown by previous studies that EXT results in a better endothelial function and in a better compliance of the vessel, meaning reduced stiffness[525]. From a molecular standpoint, it seems that HIIT improves endothelial function much better than MCT due to the greater bioavailability of NO (increase of the antioxidant status in the plasma) and reduced oxidized LDL. In addition, the activation of muscle PGC-1α (peroxisome proliferated-activated receptor gama coactivator 1α), which plays a central role for adaptive muscle metabolism responses to EXT [526] is more pronounced after HIIT.

In our study, both NO and RHI increased, although nonsignificantly, in the EXTG, which is in favour of a positive effect on endothelial function, compared to the decrease, not significant, in the CG.

Baseline RHI mean value was low, below the normal previously used cut-point for Doppler endothelial evaluation of 2[527], and for digital tonometry the value of 1.67[312], meaning endothelial dysfunction: EXTG 1.51 and CG 1.55. Baseline AI mean value was found to be -2.9 in the EXTG and -5.7% in the CG, being considered as increased stiffness. This evaluation was based on the grading of AI as follow: normal range (-10 to -30%), increased stiffness (-10 to 10%) and abnormal stiffness (>10%) [310,312].

Baseline mean NO value was determined as 14.18, in EXTG and 14.60 in CG, being normal NO measured in plasma considered in the range of 11.5-76.4 μmol/L[528]. The increase of NO in EXTG was higher, although nonsignificant (0.76 vs 0.27 in CG).

The nonsignificant variation with increase in the absolute value of AI and AI@75 was in the direction of better elasticity in both EXTG and CG. This is an important finding because we are aware that the effect on arterial stiffness reduction may favour reverse left ventricular remodeling.

Summarizing, vascular parameters obtained from peripheral arterial tonometry, AI, AI@75, RHI and NO were improved with HIIT in this sample of CRT treated HF patients,

but not enough to obtain statistical significance. Once again, we think that we would need a larger number of patients to better understand the effect of HIIT in endothelial function and arterial elasticity, which looks beneficial.

Inflammatory, apoptosis and heart failure biomarkers EXT effects in HF-CRT patients

A derangement in inflammatory factors is evident during the development of HF[199].

The prototype of inflammatory cytokine increase in HF is tumor necrosis factor (TNF-α)[529-530]. This inflammatory biomarker was remarkably decreased in patients, but only in those submitted to HIIT, after 6 months of exercise (p=0.016). The TNF-α variation differed significantly in EXTG regarding CG (p=0.008), meaning that inflammation was positively affected by the long duration HIIT and that this effect was really dependent on exercise. In the control nonexercising patients, CRT by itself had no effect in inflammation or apoptosis. As will be presented in the next chapter, also nonrandomized patients who did not undergo EXT, did not also present significant benefit of CRT regarding these mechanisms.

Serra *et al.* [531] presented evidence suggesting that chronic exercise training may improve cardiac function in adrenergically induced HF through anti-inflammatory effects. In their study, rats that had undergone a previous 12 week treadmill training programme were allocated to either isoproterenol or vehicle injection for 8 days. Sustained adrenergic stimulation caused left ventricular hypertrophy, decreased contractility and increased cardiomyocyte stiffness, as well as a proinflamatory environment, involving increased TNF-α and IL-6 expression. Regular exercise training prevented cardiac remodeling and suppressed myocardial inflammatory cytokine synthesis, suggesting a local anti-inflammatory mechanism.

There are different theories for the origin of elevation of plasmatic citokynes in HF: production and secretion by mononuclear cells, like macrophages [532], secretion by injured cardiomyocytes or by peripheral tissues, mainly skeletal muscle cells and induction of TNF-α production by lipopolyssacharides due to increased edema of the bowel wall [161].

Inflammatory citokynes, especially TNF-α are able to induce muscle wasting in end--stage HF[533] , a phenomenon due to activation of UPS (ubiquitin-proteasome system)

by MAPKS (mitogen activated protein kinases) and nuclear factor[534].

In LEICA trial (the Randomized Leipzig Exercise Intervention in Chronic Heart Failure Catabolism Study), Gielen *et al.* [535] concluded that 4 weeks of endurance training provided an excellent anabolic stimulus, with local decrease of TNF-α expression, in both younger and elderly patients with heart failure and recommended EXT as a key component of an anticatabolic treatment approach in heart failure patients of all age groups.

Other citokynes have been described to be elevated in HF, like IL-6 . In a review study of inflammatory citokynes in HF from Smart and Steel[536], it was suggested that physical therapies, employing 5 sessions of exercise per week, would be most likely to reduce serum levels of TNF-α in HF patients, differently from IL-6.

In our study, we confirmed these data with the decrease of TNF-α by exercise and the nonsignificant change of IL-6 in exercised HF patients.

Contrarily to the data of the just mentioned review and of our study, some other authors have shown the relation of exercise and IL-6 increase[537-538]. A marked increase in circulating levels of IL-6 after exercise without muscle damage has been citated in the review paper of Pedersen[537]. Plasma IL-6 was described to increase in an exponential fashion with exercise and related to the exercise intensity and duration, to the mass of muscle recruited, and to one's endurance capacity[538]. Research within the past few years has demonstrated that IL-6 mRNA is upregulated in contracting skeletal muscle[539] and that the transcriptional rate of the IL-6 gene is enhanced by exercise[540]. In spite of these findings, we did not have a significant variation of IL-6 with exercise. Also comparing this variation between EXTG and CG there was no significant difference.

Adamapoulos *et al.* [541] demonstrated that physical training, besides the significant decrease in the circulating proinflammatory cytokines TNF-α and IL-6 and their soluble receptors (TNF-RI, TNF-RII and IL-6R), caused, as well, the decrease of soluble apoptosis inducer FasL and the soluble apoptosis receptor Fas, and that these beneficial effects might be related to the training-induced improvement in functional status of patients with HF, suggesting that a persistent immune activation appears to be involved in the impaired exercise capacity characterizing this syndrome.

The other blood markers analysed in our study, including sFasL and sCD40, as IL-6, did not change significantly, neither with exercise nor with CRT only.

In our present study, BNP decreased in both randomized groups, with a tendency to significance, reflecting the benefit in HF after CRT. This variation was no statistically different between EXTG and CG, meaning that in this randomized group the effect of exercise training was not superior to the isolated effect of CRT.

Safety of HIIT Exercise in CRT-HF patients

Several years ago, exercise was contra-indicated in heart failure. After studies on cardiac rehabilitation benefits post-myocardial infarction, exercise training was expanded to HF. Studies with exercise training programs, using moderate continuous exercise documented safety, with low incidence of severe events during or after training in HF patients, if well performed[443]..

More recently HIIT, initially questioned to be potentially less safe, was demonstrated to be equally safe, since adequate patients selection and exercise supervision during exercise is provided [542].

In our patients, who were all monitored and supervised during exercise, we had during exercise no cardiac or extra cardiac complications.

Also, regarding the issue of HIIT might negatively affect reverse remodeling, we did not confirm it in our patients and, on the contrary, there was some evidence of benefit, as already discussed.

Cardiac Events at 7 months after CRT in HF patients, with and without EXT

In this sub-chapter, cardiac events at 7 months after exercise program, are discussed in all patients: total CRT sample, randomized patients for exercise and nonrandomized patients.

At 7 months after CRT (6 months after EXT), 4.95% of the HF-CRT patients died. Nonrandomized patients had the highest death rate (9.8%), while randomized patients had 2% (exercise patients had none, CG 3.5%).

Compared to the total CRT patients, those who went through an exercise program had less cardiac events than control group and nonrandomized patients.

The combined endpoint, death or HF hospitalization doubled in number of patients in the control group, compared to exercise group (4.5% EXTG vs 10.7% CG). In the total CRT patients sample, this rate was 13.8%, due to the higher rate of 19.6% in nonrandomized patients.

The second combined endpoint, death or HF hospitalization or severe arrhythmia, occurred also less frequently in EXTG than in those who did not exercise (9% EXTG vs 10.7% CG). This endpoint was also more frequent in total CRT patients (14.8%) due to nonrandomized patients rate (16.2%).

We must remember that 16% of the patients with criteria for randomization reffused to exercise, for economical or schedule unavailability and these have been added to the nonrandomized patients. Hypothetically, these patients might be less cautious with medication taking and other health procedures, compared to those who were available for exercise at once. Additionally, patients who could not be randomized, mainly for geographical reason, were different from the randomized submitted to exercise: slightly older with higher rate of female, less ischemic etiology, less atrial fibrillation, shorter QRS length and higher rate of more severe LV dysfunction.

8.2. CRT cohort study of advanced HF patients

Effects and mechanisms of CRT in Heart Failure patients

Despite the fact that the primary purpose of this thesis was to evaluate the effect of an intervalic exercise protocol in HF patients submitted to CRT, we cannot forget that these patients present the effects of CRT, which should be evaluated, as well as the involved mechanisms. We must be aware that we are comparing a potentially additional effect of exercise to the effect of CRT intervention. This fact, may even mask the isolated effect of exercise in HF, because the improvement in some parameters might even have occurred due to CRT before the effect of exercise takes place. The additional benefit of exercise will only be visible if there is no positive effect of CRT or if it significantly overpasses in magnitude the CRT positive effect. Therefore, we consider it is adequate and necessary to discuss the effects of CRT verified in this cohort sample of 121 consecutive patients.

The HF-CRT patients sample was evaluated in total and also excluding those patients (18%) who had additional exercise intervention (EXTG). The comparison between patients in the EXTG and all the others CRT patients who did not perform exercise (NEXTG) has not the same value and significance and cannot be assumed the same way as the statistical comparison among the two randomized groups (EXTG and CG), discussed in the last chapter of results. Baseline clinical characteristics, as gender, age, etiology, rhythm, severity of LV dysfunction and QRS length differed in EXTG and NEXTG, as previously remarked.

Heart Failure Responders to CRT

In this chapter dedicated to the discussion of CRT effects in the cohort population, we begin by discussing the CRT response observed. The data obtained regarding responders rate is dependent on the response definition that has been used in the study.

Echocardiographic response has been the most accepted criteria to define CRT response. Even so, different authors selected different echocardiographic criteria for defining what they considered "the best CRT response"[420], as previously explained.

The patients in this study, mostly in class III (NYHA), responded to CRT, clinically in 91 cases, 75.6%, and echocardiographically in 77 cases, 63.9%, considering as clinical response the improvement of at least 1 clinical functional class (NYHA) and as echocardiographic response the increase of at least 5% LVEF (absolute value). Looking at VO_{2p} improvement response (VO_{2p} increase >1ml/kg/min), we observed 69 functional responders (62.1%).

According to the utilized echocardiographic response criterion, we obtained a nonresponse rate of 36.1% in our population sample, which is in accordance to the literature[420].

Of course, we know that criteria of response are variable[420] and if more strict echocardiographic criteria had been used, like the decrease of more than 15% relative value for LVESVol, the number of nonresponders would had been slightly increased to 37.9%. Also, it is interesting to observe that using the functional outcome, VO_{2p}, not often used as CRT response, we found out 76 CRT responders (62.8%), a very close

number to the echocardiographic response, although those who responded by echocardiography were not necessarily the same patients who responded by exercise testing.

A large diversity regarding what defines "CRT responder" is present in the literature, as analysed by Fornwalt and coauthors[420], based on 26 CRT trials. The level of agreement among primary end points was poor, as already stated: patients defined with "positive CRT response" ranged from 32% to 91%.

It was interesting to observe in our CRT patients that simultaneous clinical and echocardiographic response, concordant response, was present only in 59 patients (48.7%), while at least one response, clinical or echocardiographically, occurred in 100 patients (82.6%). So, the number of patients with a discordant clinical/echocardiographic response was 41 (33.9%), which means that some patients felt better, but did not improve LVEF more than 5% and others, although having this LVEF improvement did not feel less symptomatic, depending on other factors, like co-morbidities and emotional status.

We are much aware that the clinical response by itself is nonspecific, largely subjective and too much dependent on different variables. Functional response, identified by VO_{2p} is dependent from the peripheral component (circulation, muscles, osteoarticular function), as well as from the central cardiac component, so more difficult to interpret as a response to CRT, by itself. On the other hand, LVEF, easily obtained, despite influenced by preload, afterload, HR, and affected by mitral regurgitation, is known to be the most widely used echocardiographic parameter and an important index of LV function. It is importantly associated to reverse remodeling, which has undoubtedly a relevant prognostic impact, as discussed before. These are the main reasons for echocardiographic criteria being the most accepted for identifying the response to CRT, in the majority of studies. Nevertheless, the best LVEF variation to recognize those who are really responding is still under discussion[420].

In our study, echocardiographic response was defined as LVEF>5% absolute improvement, in order to including most of the patients who significantly improved LV systolic function. LVEF absolute increase of 6% correspond in this LV dysfunction population to more than 17% relative LVEF increase. Using a greater LVEF increase or a

large volume decrease, would restrict the range of responders, increasing response specificity, but certainly decreasing sensitivity for beneficial response.

Comparing demographic and clinical variables between responders and non-responders in our study, there were no significant differences in the main aspects. As well, other investigators did not consider gender and etiology as independent predictors, when adjusted to LV volumes. In disagreement, some sub-analyses from randomized clinical trials, suggested that CRT beneficial effects on LV function and/or prognosis were greater in female[543] and nonischemic patients[544].

Regarding all other baseline variables, responders had significantly better ANS function, with higher SDNN, HMRe and HMRl, less dilated LV ventricules, with smaller LVED and LVES volumes and better RV function with higher TAPSE, in accordance to previous studies[545].

The responders with greater cardiac remodeling, attested by greater improvement in LVEF, LV volumes and GLS, also had less combined events: death and hospital admissions for HF or death and hospital admissions and arrhythmias.

It has to be remembered that 18% of these CRT treated patients also underwent an exercise program, which might improve response (or not), meaning that although the proportion of patients who only had the effect of CRT is high (82%), this cannot be considered as a “pure” CRT response.

We must assume it is difficult, and most of the times not possible, to compare studies with different CRT response definitions and sometimes quite different population characteristics. Once more, it is necessary and understandable to discuss the effects of CRT in this specific HF population sample, evaluated by the selected response criteria, complementing and helping to understand the results from the exercise controlled randomized trial, which is the main object of this thesis.

Clinical and quality of life functional effects of CRT in Heart Failure patients

In our total population sample of 121 HF-CRT patients (with 22 P submitted to exercise program), mean age 69.6±12.1 years, 68.5%male, 30.5 %ischemic, 74.3% in NYHA clinical function III/IV, an improvement in NYHA functional class was observed in 75.6.%

at 6 months, being the variation statistically significant ($p<0.0001$). This clinical benefit after CRT is supported in the literature since long (362). Excluding the patients who exercised, in the NEXTG, the rate of clinical response was 69%. It is interesting to notice that the rate of nonresponders in NEXTG, 31%, was more than twice superior to the 15% rate in those who were submitted to exercise. This is consistent to the better effect of exercise in clinical response verified in the RCT, with a significant different rate of clinical nonresponse in the EXTG regarding the 21.5% rate in the CG, as previously demonstrated.

In our CRT patients sample, there was no difference in this clinical benefit according to gender, age, etiology and severity of LV dysfunction. It is interesting to compare these results to those of gender-analysis performed in the patients of the Mascot study[546]. In this, despite CRT echocardiographic greater benefit in female, clinical NYHA class reduction was also not different regarding gender, as in our present study.

We know that clinical responders, defined by the improvement of NYHA class, do not always correspond to echocardiographic responders. For this reason, and because of the subjectivity of the clinical endpoint by itself for CRT response[547], translating the clinician's impression of the patient's functional limitation[548], it has been questioned several times and it is clear that it should not be used alone as an outcome measure reflecting clinical CRT response[549]. However, it is one of the most real approaches to patients' daily life, reflecting functional ability and consequently manifesting an improvement in patients' capacities and wellbeing. It should be carefully addressed by the same doctor, before and after CRT, to reduce any possible bias in questioning and interpreting the answer, though no standardization exists and inter-rater reliability is poor[550].

HF patients, besides functional symptomatic limitation, present a variety of psychosocial, socioeconomic and emotional concerns that affect their overall quality of life[551,552]. A quantitative more objective form to evaluate clinical benefit, wider in spectrum, physical and emotional, is using a questionnaire for quality of life score determination, despite all limitations and bias that might be involved[553]. Even more, self reported symptoms and quality of life have been reported to have prognostic value in HF[553,554].

Quality of life after CRT was assessed in our study by HeartQol questionnaire, an instrument also used for evaluation of quality of life in heart failure patients (491), as already described.

Quality of life improved very significantly in several previous studies: Mustic, Miracle, Miracle ICD, CARE-HF[370]. This improvement was not related to gender, contrarily to the Mustic gender analysis[362].

In our study, the significant increases of clinical functional class and HeartQol scores were maintained, even when patients submitted to exercise intervention were excluded from analysis, showing CRT beneficial effect in clinical symptoms severity and in quality of life.

Echocardiographic effects and mechanisms of CRT in Heart Failure patients

LV reverse remodeling after CRT was evaluated in our study by determination of LVEF, LV volumes, LV mass and GLS variation, as already described .

The first evidence that CRT induces reverse remodeling, was provided by Yu *et al.*, showing that LVES and LVED volumes decreased over a period of several months of CRT and that this adaptation persisted, even if pacing was temporarily suspended[407]. It is well established, nowadays, that cardiac resynchronizer implant acutely changes haemodynamic status, improving net systolic function without affecting diastolic volume [377,556]. Posteriorly at long-term, it induces LV volumes reduction, associated with an increase in LVEF and LV structural reverse remodeling, as well as improvements in mitral regurgitation severity[7,557]. Randomized trials demonstrated that these changes were associated with improved clinical outcome[7]. Whether these benefits are more specifically due to improvements in synchrony or contractile function remains unknown[558].

Our patients, at 4 months after CRT onset, had an increase in median LVEF of 9.1% and at 7 months a slightly higher increase of 11.8% ($p<0.0001$), which was already expected, since these patients had been selected to CRT for LV function improvement, according to current guidelines criteria, based on multiple studies demonstrating LV function benefit. We know well this prognostically important effect of CRT, consistently confirmed by the improvement of LVEF, the most frequently used and extensively

studied echocardiographic LV systolic function parameter, unequivocally related with mortality for long[559].

This significant increase of LVEF was maintained, when the patients submitted to exercise intervention were excluded from analysis. Analysing separately patients who did not exercise (NEXTG), we continued to observe a significant increase of LVEF and reduction of LVES volume, at 4 and 7 months, which related directly to the effect of CRT. Also, in the previously presented RCT, there was not a significant difference between exercise and control group regarding systolic LV function and volumes, except for LVES volume, whose decrease was greater in CG at 4 months, meaning that, and in accordance with the cohort results, exercise seems to have no additional effect to CRT on the magnitude of improvement of LV systolic function. We cannot state the same regarding echocardiographic response rate. At 7 months, 63.9.% echocardiographic responders were registered in the total CRT sample, in agreement with several studies[403] while 81.2% echocardiographic responders were determined in EXTG. Interestingly, echocardiographic nonresponders were superior in number in the total population (36.1%) and in the group which did not exercise (41%), compared to those who exercised after CRT device implant (18,2%), in accordance with our previous RCT analysis. This results points out, differently from the above discussed non additional effect of exercise on the magnitude of improvement of LV systolic function by CRT, to a possible additional positive effect of exercise in reducing the number of CRT nonresponders.

Regarding volumes, despite nonsignificant decrease of LVED volume at 7 months, LVES volume decreased significantly at 4 and at 7 months. In the exercise group this significant decrease was only noticed at 7 months. These volumes might continue decreasing afterwards, however the present study did not include echocardiograms later than 7 months for the detection of late responders. A recent observational study showed that half of the patients who did not respond at 6-12 months, responded until 3 years of follow-up[560].

LV mass decreased, attaining significance at 6 months, with a mean decrease of 22.85 g ($p=0.025$), as in the Miracle Study[7], although this trial had a lower mean LV mass reduction. In our study, both the EXTG (38 g of LV mass decrease) and NEXTG (18 g of LV

mass decrease) showed a tendency for reduction of LV mass, probably not reaching significance, because of the groups dimension and dispersion.

Interestingly, a more recent marker of systolic LV function, GLS, more sensitive and more independent from other factors than LVEF, improved significantly (1.079±3.871), but only at 6 months.

Few studies had examined changes in multidirectional strain, a measure of contractile function, after CRT, and the relationship with LV reverse remodeling[561,562]. Delgado *et al.* [561] demonstrated, in 141 patients with HF, that improvement in global LV strain after CRT was a long-term effect and related to the extent of LV reverse remodeling. However, significant improvement in multidirectional strain and significant reverse remodeling were only noted in responders. More recently, the MADIT-CRT investigators[369] demonstrated that contractile function, expressed by GLS, increased significantly after CRT-D compared to ICD (CRT-D: −1.4 ± 3.1% vs. ICD: −0.4 ± 2.5%, $p < 0.001$). In the CRT-D group, 78% of the patients had improved contractile function to some extent, as measured by LV GLS. Inferior improvements in the ICD-only group were likely due to optimized medical therapy.

Diastolic LV function, translated by E/e', significantly changed (E/e' ratio decreased, mean variation 3.33; p=0.039), only at 6 months, maybe reflecting that LV end diastolic pressures need more time to reduce significantly. In the Miracle study[7], which also included patients with moderate-to-severe HF, besides reduction in LV volumes and improvement of systolic function, there was an improvement in diastolic function, earlier at 3 months after CRT. However, we must keep in mind that methodology was different and in Miracle diastolic function was not evaluated by TDI, as it was in the present study.

Left ventricular reverse remodeling, demonstrated by the echocardiographic studied parameters, needed more than only 4 months to occur, being significant at 7 months, meaning that is a chronic effect of CRT[377]. Contrarly to LV remodeling, LA remodeling occurred earlier. LA volume decreased significantly, at 4 months (mean variation 20.25 ml; p=0.010), maintaining a nonsignificant decrease at 7 months. Probably, the decrease at 4 months of LA volume reflects, the more immediate consequence of initial LV mass and LVESVol reduction and LVEF increase, observed in these patients after CRT. Also, Ypenburg *et al.*[563] identified at 6 months a significant reduction of LA volume after

CRT, though non responders did not present LA reverse remodeling. Patients who did exercise did not show significant reduction in LA volume.

RA volume decreased, almost significantly at 4 months (p=0.054). Regarding right ventricular function, the parameter TAPSE increased almost significantly at 4 months (p=0.051). Both RAVol and RV function appeared to respond after 4 months, but this observation did not persist at 7 months, despite the fact that patients were on optimized therapy in the two moments.

Our hypothesis is that there was an earlier beneficial effect of CRT on the RA and RV was greater earlier due to resynchronization, but it was partially lost by progression of cardiac and/or pulmonary subjacent disease in some of the patients. We observed, as well, PSAP did not change significantly after CRT, which might be explained by the same reason. We have no respiratory tests in these patients to confirm or exclude primary or secondary pulmonary function abnormalities.

Comparing echocardiographic responders and nonresponders in all CRT patients of our study, we found that responders, besides a greater mean value decrease of LV volumes and LV mass and increase in LVEF and GLS (which are expectable), had a greater baseline TAPSE, a greater variation in SPAP, a greater improvement in clinical NYHA class and fewer composite events at 7 months.

These data, of TAPSE and PSAP variation, attest for the importance of the right-side heart influence regarding CRT response, which is defined as improvement in LV systolic function. TAPSE was found to be a predictor of echocardiographic CRT response (demonstrated in paper 1, included in attachement). In this cohort of CRT patients, echocardiographic responders also had an improvement in magnitude of clinical functional class and even more importantly in prognosis, which we know from the trials [375].

Exercise functional effects and mechanisms of CRT in Heart Failure patients

For evaluating cardiac capacity in HF-CRT patients we used, as described in the methodology, cardiopulmonary exercise testing (CPT).

CPT is an important diagnostic and prognostic tool for evaluating cardiovascular function and offers good indicators for benefit on symptoms, prognosis and ANS in HF patients.

In our patients, we observed a significant variation of VO_{2p}, already at 3 months (median increase of 1.7 ml/kg/min; p=0.02), maintaining the functional benefit at 6 months (median increase of 1.45 ml/kg/min; p=0.04). It is well known that VO_{2p} is the best parameter in CPT testing to objectively demonstrate improvement in exercise capacity, which is severely decreased, most of the times in advanced heart failure. Traditionally, it is used for risk stratification in HF patients, due to its high prognostic value[564,565].

Like VO_{2p}, some exercise functional parameters have been evaluated in the literature after CRT, but just a few times were considered as response criteria and sometimes associated to clinical criteria[420]. Patients who increase more than 10% VO_{2p} after CRT were considered by some authors as functional responders[566], while others considered all those who increased VO_{2p} at least 1 ml/kg/min, independently of the increased value[61]. In our CRT population, 76 P (62.8%) were identified as functional responders, considered those who improved VO_{2p} >1ml/kg/min. In our CRT sample, VO_{2P}, with a low baseline mean value of 14.3ml/kg/min, increased significantly at 4 and 7 months, while in EXTG it only increased significantly at 4 months. However, eventhough nonsignificant at 7 months, VO_{2p} in EXTG had a median increase of 2.18 ml/kg/min, comparatively higher than the value of 1.45±4.84 ml/kg/min, registered in the total CRT group. The explanation for nonsignificance in EXTG might be related to the smaller sample of patients who exercised.

Also, the CPT duration increased significantly at 4 and 7 months (p<0.0001 and p=0.002, respectively) and time to VAT increased significantly at 7 months after CRT (p=0.001).

In our HF patients, functional improvement after CRT, was objectively demonstrated by the increase of CPT duration, time to VAT and VO2 peak. Since the beginning of this century, core trials, like MUSTIC[362], PATH-CHF[363] and MIRACLE[7], had demonstrated the improvement of functional capacity of HF patients after CRT, through the increase of VO_{2p}.

It is interesting to notice, however, that, excluding patients who exercised and had increased CPT duration and time to VAT, at 4 and 7 months, patients with CRT effect alone (NEXTG) had only improvement in CPT duration at 4 months. Also, in the RCT, it

was demonstrated the additional value of exercise on functional capacity, with significant difference in CPT duration and time to VAT, in favour of exercise.

It is well known that VO_{2p} improvement depends on a central and a peripheral component. CRT seemed to be responsible for the central component improvement and HIIT additionaly was also responsible for the sustained peripheral component improvement. In this cohort, it looks that functional capacity improvement was attributed mainly to exercise after CRT. It looks understandable that in very deconditioned patients, the resynchronizer implant will not be enough to improve the peripheral component, namely the skeletal muscles function.

Another CPT parameter, VE/VCO_2 slope, with a high baseline median value of 37.9, decreased significantly at 4 and 7 months after CRT ($p<0.0001$ and $p=0.003$, respectively). The variation of this parameter maintains significant even excluding the exercise patients, who had already no significant variation of VE/VCO_2 slope in the analysis of RCT.

We know that the rate of ventilation per unit increase of carbon dioxide (VE/VCO_2) in HF is higher than in normals individuals. Patients with slopes above the upper limits of normal have poorer exercise tolerance and worst prognosis[565, 567].

Both, VO_{2p} and $VEVCO_2$ slope, show good correlation to prognosis, with equivalent prognostic power and complementary prognostic information[564]. The baseline VO_{2p} and $VEVCO_2$ slope mean values of our patients were definitely abnormal, correlating to bad prognosis. CRT described effects are expected to associate with a better prognosis in those patients who improve VO_{2p} and/or decrease $VEVCO_2$ in posterior follow-up, which was not a purpose of evaluation in this study.

In CPT, we also evaluated heart rate recovery at different moments of recovery, min1 (HRR1) and min 6 (HRR6), after exercise.

It is recognized that, as HRV analysis in 24-hours Holter, HRR in CPT is clinically used in noninvasive assessment procedures for the determination of cardiovascular parasympathetic function[568]. Heart rate recovery after graded exercise is one of the commonly used techniques, reflecting autonomic activity and predicting cardiovascular events and mortality, not only in cardiovascular system disorders, but also in various systemic disorders[568].

During exercise, the heart rate increase is due, in part, to a reduction in vagal tone and, in part, to an increased sympathetic activity. Recovery of the heart rate immediately after exercise is a function of vagal reactivation combined to sympathetic withdrawal. A generalized decrease in vagal activity is known to be a risk factor for death and CV disease progression, so consequently the heart rate after exercise is an important prognostic marker[569].

A delay in HRR, indicative measure of reduced parasympathetic activity, has been observed before in patients with chronic heart failure[568]. In addition, a decrease in parasympathetic and/or an increase in sympathetic HRV indexes have also been described in patients with cardiovascular disease[569].

In our population, baseline and maximal heart rate did not change significantly, however the variation of HR at 1st minute and 6th min recovery regarding the peak HR, were significant, but only at 6 months after CRT. The improvement of HHR1 (p=0.015) and HHR6 (p=0.03), is in support of the modulating effect of CRT on the autonomic nervous system, as referred in other studies[568,570], which does not look to be immediate and needs more than 3 months. Excluding those patients who did the exercise program, the significant improvement maintains.

This might lead us to the conclusion that CRT, modulating ANS, induces a positive peripheral effect on the CV system in advanced heart failure patients, as might be responsible for the beneficial central effects, of reverse remodeling, already discussed. It is not immediate and takes some months to have an ANS modulation effect after CRT device implant. In our sample, exercise did not add benefit to CRT, regarding these mechanisms.

Imaging autonomic system function effects in Heart Failure patients

Imaging of sympathetic inervation has been performed through 123-MIBG-Cardiac scintigraphy. As previously mentioned and explained in this chapter, the parameters derived from the scintigraphic image, HMRe, HMRl and WOR, with known prognostic value in HF[77], have also been demonstrated to have the same value after CRT (67).

In our total population, we found nonsignificant variation of HMRe, HMRl and WOR after CRT.

HF patients selected to CRT have different etiologies. Evaluating separately nonischemic cardiomyopathy patients, we found that HMR late and WOR correlated significantly with CRT echocardiographic response. In this nonischemic group of CRT patients, those with baseline HMRl >1.5 had a 2-fold greater possibility of being an echocardiographic responder (FEVE>5%), compared to those with HMRl<1.5. This association of HMRl and LVEF>5% was confirmed by multivariate analysis (p=0.053).

Resuming, these results indicate that advanced HF nonischemic patients with less innervated hearts were less prone to respond echocardiographically to CRT.

A few studies have been performed regarding sympathetic imaging after CRT. According to Burri *et al.*[571], who studied a smaller sample of 16 patients, changes in cardiac adrenergic activity after CRT, were noticed at 9 months, a later period of evaluation regarding our study. They investigated whether these changes were related to LVEF improvement, showing that responders only had lower ^{123}I-MIBG washout at follow-up compared with non-responders, indicating improvement of cardiac sympathetic nerve activity after CRT in responders (p=0.036). A moderate correlation between increase in LVEF and decrease in ^{123}I-MIBG washout was also demonstrated (r=0.52, p=0.04), concluding that CRT induced a reduction in cardiac sympathetic nerve activity in responders, that paralleled the improvement in LVEF, whereas non-responders did not show any significant changes.

Another study[572] evaluated, by ^{123}I-MIBG cardiac scintigraphy, 30 HF patients, before and at 3 months after CRT, and correlated these data with CRT clinical response. The HMR and WOR were associated with CRT response (p=0.005 and p =0.04, respectively). The HMR ratio was the only independent predictor of CRT response (p=0.01), with an optimal cut-off point of 1.36 (sensitivity 75%; specificity 71%). They concluded that the improvement in autonomic nervous system activity correlated with a positive clinical CRT response. Lower ^{123}I-MIBG uptake before therapy was associated with CRT nonresponse. The HMR could be helpful in selecting patients for CRT, at least in some doubtful cases.

With a similar conclusion, Cha *et al.* [573] evaluated 45 HF patients with CRT- defibrillator, at baseline and after implant (3 and 6 months), with ^{123}I-MIBG scintigraphy. After CRT,

NYHA class and LVEF improved. Along with improvement in SDNN and SDANN, HMR increased (1.82±0.58 vs 1.97±0.59; p=0.03), whereas the mean washout rate was reduced (48%±19% vs 37%±22%; p=0.01). Compared with nonresponders, responders had a higher HMRl (2.11 vs 1.48; p=0.003) and lower washout rate (37% vs 62%; p=0.003) at baseline. These authors concluded that CRT improved sympathetic function, assuming that cardiac sympathetic reserve may be a marker for the reversibility of the failing myocardial function.

The two previous studies showed an association of low cardiac inervation with CRT nonresponse, which is very similar to our results in the nonischemic HF patients.

Other authors [574], also evaluated nonischemic HF patients, 37 P, who underwent CRT, before and 7 months after CRT. In responders (≥15% absolute decrease in LVES Vol) the mean HMRl was significantly increased ($p<0.05$) and serum levels of hs-CRP were decreased ($p<0.01$). Such improvements were not observed in nonresponders. Stepwise multiple regression analysis showed that the reduction in hs-CRP level was independently associated with the increase in the HMRl. These authors demonstrated that cardiac sympathetic nervous dysfunction and systemic inflammation were both improved in nonischemic HF patients responders to CRT. Furthermore, the reduction in systemic inflammation was associated with the improvement in cardiac sympathetic nervous dysfunction. Contrarily, in our study, we did not demonstrate in this 121 patients sample an increase in HMRl, nor a reduction of inflammation after CRT. We may hypothesize that with a longer period after cardiac resynchronizer implant we might had obtained an improvement in autonomic nervous system and possibly in inflammatory parameters, at least in some subgroups of these patients, namely in nonischemic cardiomyopathy.

Endothelial effects of CRT in Heart Failure patients

Benefits on endothelial function after CRT have been demonstrated, which is prognostically important[401].

In our study, the endothelial studied parameters, RHI, AI and AI@75, did not vary significantly after CRT. Also, NO variation did not reach statistical significance.

Nevertheless, RHI baseline mean value was low (1.58), indicating endothelial dysfunction and increased after CRT to a mean value superior to 1.67, the cut-point of normality for endothelial function by RHI, which may have an impact on prognosis.

Inflammation, Apoptosis and HF biomarkers effects in Heart Failure patients

CRT did not present considerable beneficial effects on markers of inflammation and apoptosis in the total CRT population sample. We even observed a significant increase of CD40 at M2, which disappeared at M3. BNP and hs-CRT decreased but nonsignificantly.

It is known that the effect of cardiac resynchronization therapy (CRT) on systemic inflammation change associated with heart failure is not well characterized[575-577]. Some studies[575], showed the lack of effect after 3 and 12 months of CRT therapy on plasma levels of inflammatory markers such as TNF-α and IL-6, as happened in this sample of patients CRT treated HF patients.

Contrarily, as already described, others observed that CRT had a positive effect on inflammation, namely in reducing hs-RCP level[578].

Effects in patients with CRT without Exercise

As already explained, besides the analysis performed regarding exercise effects after CRT in the randomized controlled trial, we decided to evaluate the isolated effect of CRT in the patients who did not exercise. In this evaluation, despite the non randomization, we enlarged the group of patients with CRT who did not do any exercise program.

Just to summarize, what has been already reported and debated along the discussion chapter (cohort study subchapter), the results in the randomized controlled trial are not comparable to those in the cohort study. A greater proportion of female and nonischemic were present in the group of patients who did not exercise (NEXT).

Patients who did not exercise, had at 7 months (M3) after CRT a significant increase of NYHA, LVEF and Heartqol scores, as exercise patients. Differently from these, people who did not exercise had GLS and VE/VCO_2 significantly improved.

Also, inflammatory and apoptosis markers did not have significant variation, they increased in patients who did not exercise, while in the EXTG there was a significant decrease of TNF-α, calling attention, once more, for the effect of exercise on inflammation as an additional beneficial mechanism to CRT.

Comparatively to those who exercised, the NEXTG had an inferior number of clinical (71% NETXG vs95% EXTG; p=0.017) and echocardiographic responders (69.7% NEXTG vs77.3% EXTG; p=ns) and less improvement in clinical functional class and in functional capacity, with inferior CPTdur (p=0.001) and VATtime (p=0.000).

We must be aware that these results are only based on an observational study, without a homogeneous population with EXT, differently from the RCT, nevertheless they were quite similar in conclusions to those found in the randomized clinical trial.

9. LIMITATIONS AND STRENGHTS

The strengths of this thesis, in order to respond to our hypothesis, are here described.

Strenghts: the large sample size of advanced HF patients submitted to CRT, exhaustively studied, enrolled in one single-center, which assures uniformity in patients evaluation and treatment; the great number of variables, measured before and after resynchronization, mostly including two moments after CRT (4 and 7 months); the use of a specific and innovative intervalic high intensity exercise training protocol (HIIT), hypothetically better for central cardiac improvement, for long duration intervention, uniformly applied to advanced heart failure patients treated by CRT, allowing to study not only the additional effects of HIIT for a period of 6 months and effects of CRT for 7 months, but also the mechanisms for exercise and CRT intervention in heart failure; the possibility to evaluate in-hospital, under continuous monitoring and surveillance, the potential limitations and complications of exercise training, mainly HIIT, in these subsets of patients and the prognosis at 7 months after CRT.

Limitations: the small number of patients who were randomized and accepted to perform HIIT, though in the literature, by the time this study was done, there were just a few studies with HIIT in HF-CRT patients, with very small numbers, inferior to ours; the number of exercise sessions, once the initial HIIT protocol was planned to include 3 sessions per week on alternate days, however finally only two sessions per week were considered, because of patients' different kinds of limitations, namely economical and job-related, which could compromise participation and adherence.

Even so, despite the limitations, it may allow to understand the effects of this type and volume of exercise training in this very selected group of advanced HF-CRT patients.

10. CONCLUSION

In this randomized controlled trial, evaluating a high intensity interval training exercise protocol for 6 months in a sample of patients with advanced HF, with severe LV systolic dysfunction and multiple etiology, selected for CRT according to the current guidelines, the exercise intervention demonstrated to add benefit to CRT and to be safe, without major complications resulting from the exercise program.

Clinical functional improvement had a greater magnitude in patients who exercised. The proportion of clinical and echocardiographic responders was greater in the group of exercise, with identical improvement in quality of life.

The functional capacity improvement benefit was greatly dependent on HIIT, as demonstrated by the prolonged CPT duration and time to VAT.

The HIIT effect on reverse remodeling, was strongly suggested by the occurrence of less echocardiographic nonresponders and by the LV mass decrease of more than 3-fold, regarding the CG, at 6 months, in the EXTG. This effect did not reach statistical significance, most probably due to the dimension of the sample. Increasing the number of patients or prolonging the exercise program, possibly would increase the magnitude of the effect and might confer statistical significance to this positive modification.

Regarding pathophysiologic mechanism inflammation, which play a central role in the aggravation of HF, this was positively influenced by exercise, illustrated by the significant decrease of TNF-α only after 6 months of HIIT.

Additionally to the RCT, in the cohort study, which included all the CRT treated HF patients in the sample, with and without exercise, beneficial effects on quality of life, LV reverse remodeling and functional capacity were also observed after CRT. Mechanisms involved, like ANS dysfunction, were linked to the improvement observed in nonischemic DCM after CRT, with less denervated heart patients responding more to resynchronization regarding the improvement of LV systolic function.

Finally, major cardiac events at the end of the exercise training program, at 7 months after CRT implant, including death and hospitalization, were inferior in number relatively to those occurred in patients who did not exercise.

Globally, the results of this thesis, suggest frankly, that exercise training treatment should be added, after CRT, as a protocol, in the modality of HIIT, whenever possible, at least twice a week, 60 minutes sessions, ideally maybe 3 times a week, including resistance (strength) training, in order to decrease the number of clinical and echocardiographic nonresponders, with additional benefit on symptoms, exercise functional capacity and inflammation, possibly decreasing the number of cardiac events. Additionaly HIIT did not exert deleterious effect on cardiac remodeling and, on the contrary, the present results point to a possible benefit on reverse remodeling. Even more, considering that CRT is an expensive therapy aimed to benefit selected HF patients, but knowing in advance that more than 30% will not respond, it looks mandatory to use an easily available, unexpensive, nonpharmacologic therapy as exercise, which only needs unexpensive tools, physical space, an organized team and adherent patients to reduce the number of CRT nonresponders.

Larger series with HIIT in HF-CRT patients will be needed to confirm these results.

11. FUTURE DIRECTIONS

Next studies will be needed to address issues like, whose patients will benefit most from HIIT exercise after CRT, what is the best exercise training protocol for these CRT patients, how can we increase adherence to exercise, if the increase of HIIT program duration modify results, should strength training be increased and what is the best intensity and modality exercise protocol for these patients.

Also, identifying which are the best parameters to define non CRT responders and what is the best way of identifying response to CRT, will be important.

The results obtained in this sample and the possible need for longer exercise program and longer evaluation (1 year) needs to be confirmed

in a larger population.

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13.ATACHEMENTS