UNIVERSITAT DE BARCELONA

Muscle dysfunction and exercise-based cardíac rehabilitation in cardíac diseases:

From muscular physiology in animals to prognosis impact on patients

Ignacio Alfredo Cabrera-Aguilera

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MUSCLE DYSFUNCTION AND EXERCISED-BASED REHABILITATION IN CARDIAC DISEASES

FROM MUSCULAR PHYSIOLOGY IN ANIMALS TO PROGNOSIS IMPACT ON PATIENTS

by

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in fulfillment of the requirements for the degree of

Doctor of Philosophy

Programa de Doctorado en Biomedicina

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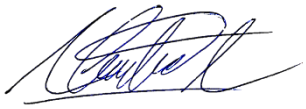
Barcelona, Spain

2022



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Pre-doctoral fellowship:

Chilean Doctorate Fellowship 2017 – 72180089. Agencia Nacional de Investigación y Desarrollo. Ministerio de Ciencia, Tecnología, Conocimiento e Innovación. Gobierno de Chile.

A mi Madre Marta

A mi Hermana Daniela

A mi Padre Alfredo

A mi familia, a mis amigos y amigas

AGRADECIMIENTOS

Llegar a este momento de escribir agradecimientos me permiten reflexionar, por una parte, lo que formó parte de la experiencia académica-profesional, y por otra, sobre las contradicciones de humano *sentipensante* que me genera ir culminando una parte del proceso después de algunos años como migrante lejos de casa, donde las y los amigos, se vuelven la familia.

Las maravillosas amistades – tanto dentro como fuera de los laboratorios – que se han ido construyendo durante mi residencia por Catalunya en los últimos pandémicos años me hacen sentir desbordado de fortuna y también de agradecimiento. Es difícil cuantificar y medir estas cosas, pero tengo la certeza de que, si fuera a través de la estadística, de seguro sería estadísticamente *significativa* la probabilidad de haber coincidido con las personas con quienes pude compartir durante el desarrollo del trabajo que sustenta este documento.

Así como la confección de esta Tesis y los artículos que la componen, mi paso por Barcelona continúa siendo una experiencia colectiva. Los frutos no son producto de un esfuerzo único y personal, sino por el contrario, es el resultado de una sumatoria de quehaceres en equipo, que comienza por los supervisores de este trabajo.

Quiero comenzar, sobre todo, mostrando mi profunda admiración y gratitud a mi directora de Tesis Núria Farré por la confianza entregada, por inspirarme la autonomía como investigador, por el acompañamiento en el camino científico-académico, por la constante paciencia en cada construcción y deconstrucción de ideas y por todas las enseñanzas. Las médico-científicas y las no tanto.

Al profesor Ramon por abrir las puertas de su laboratorio, por mantener siempre – y desde el inicio- la preocupación de que el desarrollo de esta Tesis se mantuviera relacionada con mi *background* como Kinesiólogo (Fisioterapeuta aquí en este territorio) y por la confianza entregada en cada tarea y proyecto.

A las enseñanzas adquiridas por Marta Torres con el isoflurano y la anestesia, a Isaac Almendros en el estabulario, a Bryan Falcones con los ventiladores mecánicos, a Laia Yañez con las bombas, a Esther Marhunda con las pipetas, a Alvaro Villarino con los reactivos, a Ignasi Jorba con MatLab, a Jair con la programación, a Jordi Otero con el Aurora, a la Eli con el autoclave, a Marta Tajes con los tejidos y a Miguel Rodríguez por ilustrarme constantemente con su infinita capacidad para resolver problemas. Es mucho más lo que me han enseñado, pero agradezco nuevamente la paciencia de cada uno con este curioso sujeto. Gracias por la disposición de todo este tiempo.

A todo el equipo técnico, médico, profesional y administrativo del grupo de investigación Heart Diseases Biomedical Research del IMIM, de la sección de Cardiología del Hospital del Mar y de la Unidad de Rehabilitación Cardiaca y prevención Cardiovascular: A la profesora Sonia Ruiz, a Neus Badosa, Marta Tajes, Lluis Recasens, Diana Mojón, Miren Vicente, Alicia Calvo, Begoña Benito, Consolació Ivern, Marc Llagostera, Ester Marco, Luís Salas. Su trabajo y profesionalismo en el quehacer diario no sólo dieron paso a las investigaciones clínicas

realizadas - mientras la pandemia amenazaba la investigación clínica del desarrollo de esta Tesis - sino que, además, han influido positivamente en mi manera de entender la salud y en los deseos de querer continuar vinculado a la investigación en cardiología.

A mis amigos y amigas más cercanos por acompañarme en cada parte del proceso de crecimiento personal y científico. Y también aquellos que, de alguna manera u otra, lograron revindicar y/o resignificar simbólicamente cosas y palabras en mi vida: a Juan Pino, Pía Rodríguez, Joan Acunya, Pamela Verdugo, Pedro Marchant, Oriana Jofré, Rodrigo Torres, Núria Soler, Sergio Echeverría, Paloma Correa, Sebastián Zagmutt, Francisco Jiménez, Yeny Gamboa, Gonzalo Latin, Nara Agaccino, Ismael Canales, Prissilla Soto, Bryan Falcones, Sara Ferrari, Héctor Sanz, Amélie Houdet, Felipe Pérez, Alejandra Leyton, Rodrigo Ibarra y seguro que otros y otras que ahora mismo no tengo tan presente.

A los amigos y compañeros del Club Social y Deportivo Chicha Fresca, a las compañeras del espacio sociocultural y creativo La Caprichosa y al Grupo de Guías y Scout Lola Anglada.

A *Los Brothers*, los amigos de "toda la vida". Esos que hacen que las distancias y el tiempo entre Chile y España, no signifiquen demasiado: Chiri, Mota, Xime, Alvaro, Coni y Nico.

A la Agencia Nacional de Investigación y Desarrollo del Ministerio de Ciencia, Tecnología, Conocimiento e Innovación del Gobierno de Chile, por la adjudicación de beca doctoral para realizar las investigaciones incluidas en esta memoria.

Finalmente, a mi familia. A mi sobrino Amaro por esperar pacientemente. A mi hermana Daniela, a mi madre Marta y a mi padre Alfredo, por vuestro apoyo incondicional durante no sólo el desarrollo de este trabajo, sino que durante estos 30 años.

A Ustedes les agradezco y les dedico esta Tesis.

Barcelona, 17 de agost de 2022

Núria Farré López, cap de secció de la Unitat d'Insuficiència Cardíaca, servei de cardiologia, Hospital del Mar, i directora de la Tesi Doctoral del Sr. **Ignacio Cabrera Aguilera**,

INFORMA, sobre la participació personal del Sr. **Ignacio Cabrera Aguilera** en els articles científics inclosos a la seva Tesi Doctoral, que es presenta en el format de compilació d'articles. A continuació es fa referència als articles en el mateix ordre en que apareixen en la memòria de la Tesi doctoral:

1. **I. Cabrera-Aguilera, B. Falcones, A. Calvo-Fernández, B. Benito, E. Barreiro, J. Gea, R. Farré, I. Almendros and N. Farré, "The conventional isoproterenol-induced heart failure model does not consistently mimic the diaphragmatic dysfunction observed in patients". PLoS One., vol. 15, pp. 1–17,. Jul 2020. IF = 3.24, Q2/T2 (26/72).**

Aquest és el treball nuclear de l'apartat experimental translacional de la Tesi Doctoral delSr. Ignacio Cabrera Aguilera. El doctorand va ser responsable de tot el desenvolupament experimental, inclòs el model animal. Va posar a punt el sistema de mesurament *ex-vivo* de la funció muscular ajustant *hardware* i participant directament en el desenvolupament, conceptualització i disseny del *software* per al protocol d'estimulació electromuscular. Va efectuar l'anàlisi estadístic dels resultats i va a realitzar totes les mesures i càlculs referents a la funció muscular. Va contribuir directament en les mesures experimentals d'ecografia i qPRC. Va tenir un paper protagonista en la producció i redacció de l'article. Aquest article no s'ha utilitzat ni formarà part, implícita o explícitament, de cap altra tesi doctoral.

2. **I. Cabrera-Aguilera, C. Ivern, N. Badosa, E. Marco, L. Salas-Medina, D. Mojón, M. Vicente, M. Llagostera, N. Farré and S. Ruiz-Bustillo, "Impact of and Reasons for Not Performing Exercise Training After an Acute Coronary Syndrome in the Setting of an Interdisciplinary Cardiac Rehabilitation Program: Results From a Risk-Op-Acute Coronary Syndrome Ambispective Registry". Front. Physiol., vol. 12, pp. 1–8, Nov. 2021. IF = 4.755, Q1/T1 (20/81).**

El Sr. Ignacio Cabrera Aguilera s'ha encarregat de fer el seguiment de tots els participantsde l'estudi, incloent-hi la corresponent recol·lecció de dades així com la seva posterior processament i anàlisi. Ha contribuït substancialment a la confecció de la base de dades i ha tingut un paper protagonista en la producció i redacció de l'article. Aquest article no s'ha utilitzat ni formarà part, implícita o explícitament, de cap altra tesi doctoral.

3. **I. Cabrera-Aguilera, C. Ivern, N. Badosa, E. Marco, X. Duran, D. Mojón, M. Vicente, M. Llagostera, N. Farré and S. Ruiz-Bustillo, "Prognostic Utility of a New Risk Stratification Protocol for Secondary Prevention in Patients Attending Cardiac Rehabilitation". J. Clin. Med., vol. 11, pp. 1910–21,. Mar 2022. IF = 4.964, Q2/T1 (54/172).**

Utilitzant la base de dades de l'estudi previ, el doctorand ha actualitzat les dades de la capacitat cardio-respiratòria per fer l'estratificació de tots els pacients inclosos en aquest estudi. El Sr. Ignacio Cabrera Aguilera s'ha fet responsable de la recol·lecció de dades i l'anàlisi de resultats. Va a contribuir directament en la conceptualització, metodologia, en l'anàlisi formal i ha tingut un paper protagonista en la producció i redacció de l'article. Aquest article no s'ha utilitzat ni formarà part, implícita o explícitament, de cap altra tesi doctoral.

A més, cal destacar que el Sr. Ignacio Cabrera Aguilera ha col·laborat molt activament amb altres treballs de recerca dels dos laboratoris en què ha estat durant la realització de la sevaTesi Doctoral, com queda reflectit en la resta d'articles on el doctorand és autor principal o coautor, tal i com s'exposa al capítol "Contribution to other research projects, publications and conference communications" de la memòria de Tesi.

Així ho faig comptar pels efectes que siguin pertinents davant la corresponent Comissió de Doctorat de la Universitat de Barcelona.

Firmado por NURIA FARRE LOPEZ - DNI 46460286X el día 17/08/2022 con un certificado emitido por EC-Ciutadania

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ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
A-NT	Accept – not training
A-T	Accept- training
ATP	Adenosine triphosphate
BMI	Bodi mass index
BW	Body weight
CAD	Coronary artery disease
CHF	Chronic heart failure
CIF	International classification of functioning
CPR	Cardiopulmonary resuscitation
CR	Cardiac rehabilitation
CRP	Cardiac rehabilitation program
CSA	Cross-sectional area
CV	Cardiovascular
CVD	Cardiovascular diseases
DOX	Doxorubicin
EB-CRP	Exercised-based cardiac rehabilitation program
EC	Exercise compliance
EGG	Electrocardiogram
ET	Exercise training
FE	Ejection fraction
FG	Fast glycolytic
FIG	Fast intermediate-glycolytic

FOG	Fast oxidative-glycolytic
FS	Fraction shortening
HF	Heart failure
HW	Heart weight
Hz	Hertz
IHD	Ischemic heart disease
ISO	Isoproterenol
L	Low
LAD	Left anterior descending artery
L-C	Low risk – control
L-E	Low risk – exercise
LV	Left ventricle/ar
LVEDD	Left ventricular end-diastolic
LVEF	Left ventricular ejection fraction
LVESD	Left ventricular end-systolic
METS	Metabolic equivalents
MyHC	Myosin heavy chain
MI	Myocardial infarction
NL	No-low
NL-C	No-low-control
NL-E	No-low-exercise
Non-STEMI	Non-ST-elevation myocardial infarction
PCC	Patient-centered care
Ph.D.	Doctor of Philosophy
Pi	Phosphate
QoL	Quality of life
R-NT	Reject - not training

RS	Risk stratification
RV	Right ventricle/ar
SDI	Strength decrement index
SNA	Autonomic nervous system
SO	Slow oxidative
SSC	Spanish society of cardiology
STEMI	ST-elevation myocardial infarction
UA	Unstable angina
WHO	World health organization

Chapter I:
Introduction

1 Cardiovascular diseases

Cardiovascular diseases (CVD) are a general term for conditions affecting the heart or blood vessels and cover most diseases in both cardiovascular and cerebrovascular systems. There are four types of CVD: coronary artery disease (CAD), stroke, peripheral arterial disease, and aortic disease. Despite sustained declines in CVD mortality in many countries across Europe, CVD have remained the most common cause of death within the region and the leading cause of death and morbidity in the developed world (1). Significantly, heart diseases contribute to more than half of all CVD deaths (2). The most common heart diseases are ischemic heart disease (IHD) or CAD, including acute coronary syndrome (ACS) and heart failure (HF).

1.1 Acute coronary syndrome

CAD is a pathological process characterized by atherosclerotic plaque accumulation in the epicardial arteries, whether obstructive or non-obstructive. This process can be modified by lifestyle adjustments, pharmacological therapies, and invasive interventions designed to achieve disease stabilization or regression. The disease can have long, stable periods but can also become unstable at any time, typically due to an acute atherothrombotic event caused by plaque rupture or erosion. However, the disease is chronic, most often progressive, and hence severe, even in clinically silent periods. The dynamic nature of the CAD process results in various clinical presentations, which can be conveniently categorized as either acute (ACS) or chronic coronary syndromes (3).

The clinical presentation of ACS is broad. It includes cardiac arrest, arrhythmias, heart failure, cardiogenic shock, or mechanical complications. The leading symptom initiating the diagnostic and therapeutic cascade in patients with suspected ACS is acute chest discomfort, described as pain, pressure, tightness, and burning. Chest pain-equivalent symptoms may include dyspnea, epigastric pain, and pain in the left arm. Based on the electrocardiogram (ECG), at least two groups of patients should be differentiated: non-ST-elevation myocardial infarction (non-STEMI) and ST-elevation myocardial infarction (STEMI) (Figure 1) (4,5).

ACS is the collection term for STEMI, non-STEMI, and unstable angina (UA). The pathological correlate at the myocardial level is cardiomyocyte necrosis or, less frequently, myocardial ischemia without cell damage (unstable angina). UA is chest pain or discomfort

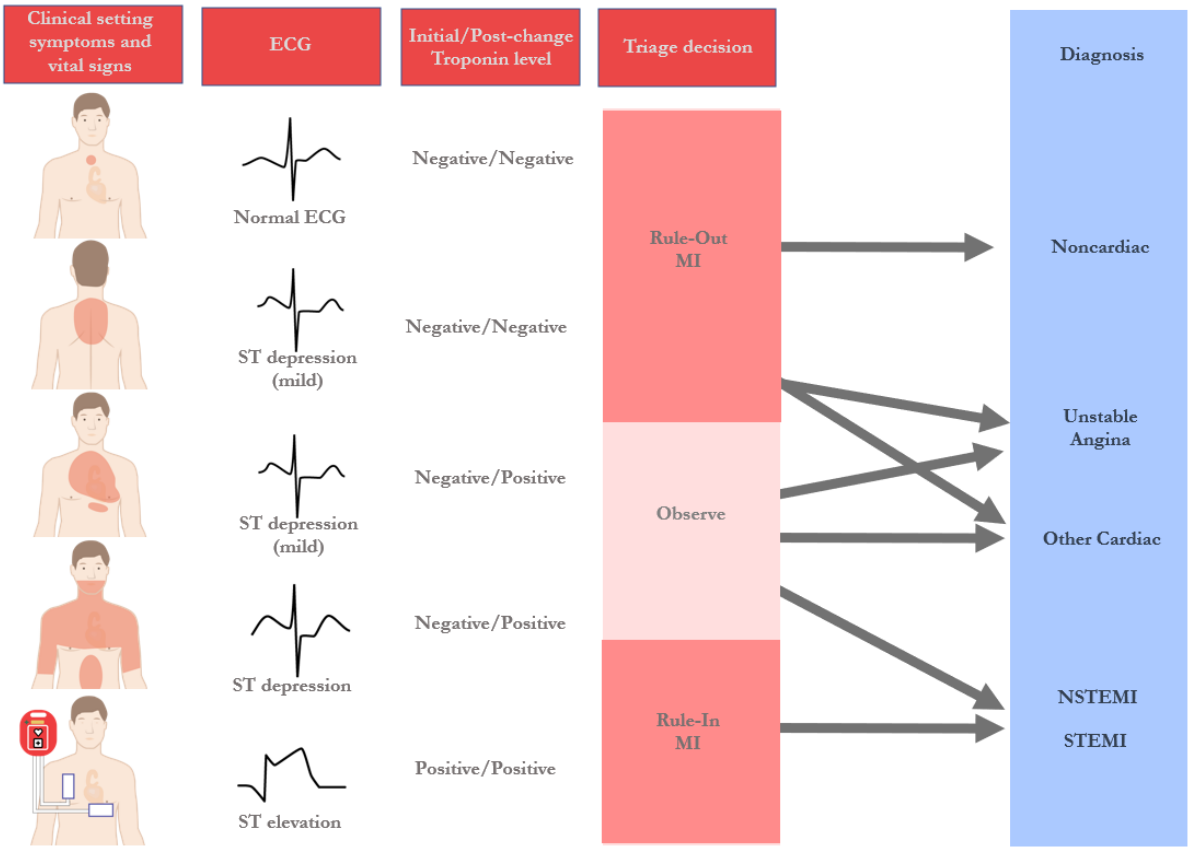

Figure 1. Diagnostic algorithm and triage in acute coronary syndrome. Adapted from ESC guidelines (5).

ECG = electrocardiogram/electrocardiography; MI = myocardial infarction; NSTEMI = non-ST-segment elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction

that is accelerating in frequency or severity and may occur while at rest but does not result in myocardial necrosis. The discomfort may be more severe and prolonged than typical stable angina. UA, NSTEMI, and STEMI share common pathophysiological origins related to coronary plaque progression, instability, or rupture with or without luminal thrombosis and vasospasm (1,6). Some of these populations may present with ongoing myocardial ischemia, characterized commonly by one or more of the following: recurrent or persistent chest pain, marked ST-segment depression on 12-lead ECG, hemodynamic or electrical instability, and HF. This clinical context is one of the leading causes of HF (4,7).

1.2 Heart failure

HF is a clinical syndrome characterized by reduced cardiac output and/or elevated intracardiac pressures during stress or at rest, leading to exercise intolerance (8). HF is not a single pathological diagnosis. It is a clinical syndrome consisting of cardinal symptoms such

as breathlessness, ankle swelling, and fatigue that may be accompanied by signs such as elevated jugular venous pressure, pulmonary crackles, and peripheral edema (7).

Despite the evident progress in the treatment of CVD over the last decade, the incidence and high prevalence of HF are expected to increase worldwide due to obesity epidemics and population aging (10,11). In older adults, there is an inter-relationship between aging-frailty and HF. Some hemodynamic and perfusion consequences associated with HF may exacerbate this relationship (Figure 2) (9).

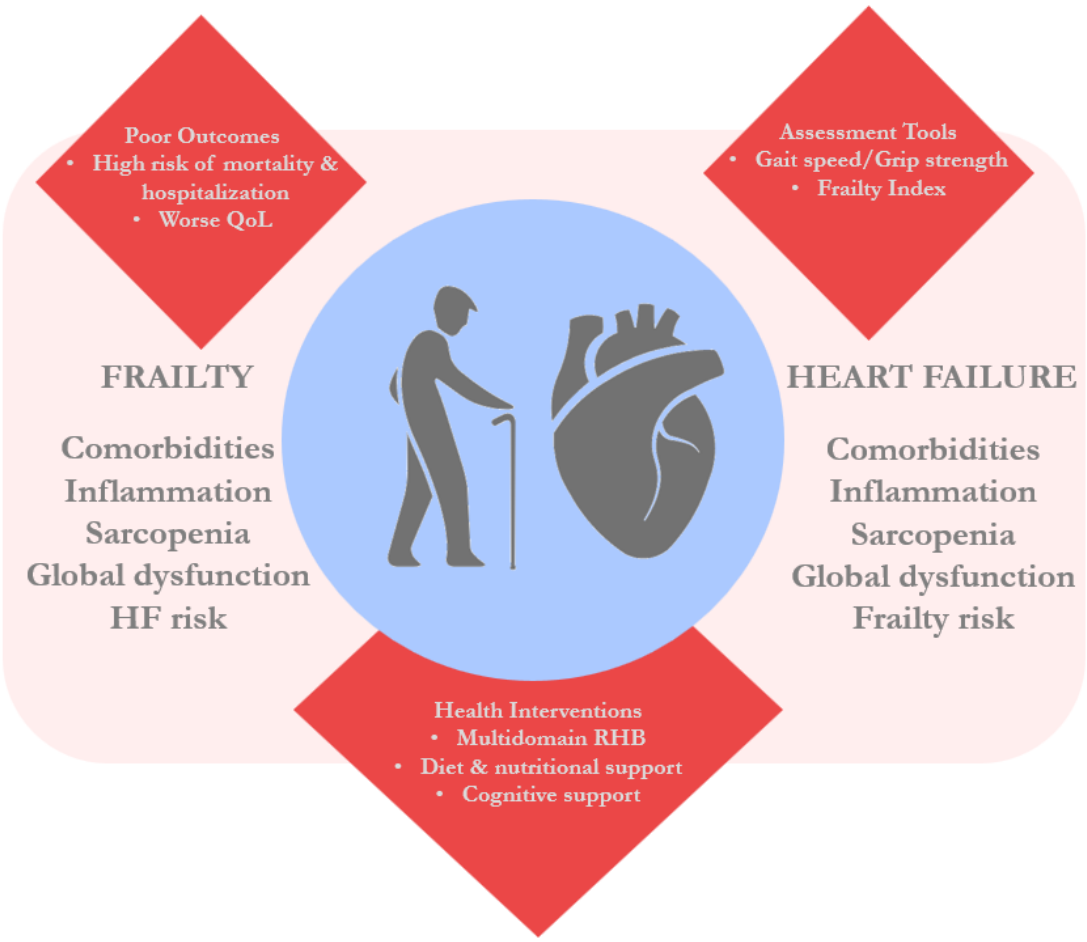

Figure 2. The inter-relationship between frailty and heart failure. Adapted from Pandey and cols, 2019 (9).

QoL = quality of life; HF = heart failure.
Frailty and heart failure share common pathological mechanisms, often coexist and are associated with worse clinical and patient-oriented outcomes. Identifying and targeting frailty in HF patients are essential since multi-domain interventions improve outcomes.

HF can develop in patients with a wide range of left ventricular ejection fraction (LVEF) (8). HF with preserved LVEF (HFpEF) represents approximately 50% of patients with HF and is the main form of HF in the aging population. It has recently received attention in translational and clinical research (9,12,13).

1.3 Epidemiology

Less than ten years ago, CVD were the leading cause of death, with an estimated 17.5 million deaths annually, representing 31% of global deaths with 7.4 million attributed to CAD (14). CAD alone contributes to 1 of every 7 deaths in America and 20% of all deaths in Europe (15,16). CAD is a major cause of physical disability, particularly in the rapidly growing population of elderly persons (17). It is the most common form of heart disease and is one of the leading causes of HF.

Despite advances in treatment, HF remains one of the main causes of hospitalization, readmissions, death, and disability worldwide, with a well-known impact on quality of life (QoL) (18–20). HF is a common cardiovascular problem in Western countries, affecting 2% of the adult population and increasing to 10% in those over 65 years old. In Spain, 68% of the patients with HF are over 75 years old and 30% are over 84 years old (11,21,22). These numbers may increase due to aging, improved of survival rates, and expanded life expectancy.

1.4 Prognosis

Reports over the last years have shown that prevalence is increasing due to the growing number of patients living with these CVD, and mortality rates are decreasing. Despite new pharmacological and non-pharmacological treatments and devices, long-term prognosis in IHD and HF remains poor. Moreover, quality of life is significantly reduced due to, among other factors, worsening exercise tolerance, decreased cardiorespiratory capacity, and muscle weakness, which can lead to reduced physical function, and impaired mental health (5,7,23-28).

The consequences on quality of life and social participation resulted in the inclusion of cardiac diseases by two international entities in two categories. The *International Classification of Functioning, Disabilities and Health* (CIF) and *World Health Organization* (WHO), through the categories of *Body Functions and Structures* and *Activities and Participation*, have left behind the idea that dysfunctions and disability are purely medical, biological and structural conditions. In 2001 these institutions prescribed disability and health from a biological, individual, and social perspective, including cardiac diseases, and highlighted the importance of cardiac rehabilitation (29–31).

Fortunately, from a clinical perspective, all these complications and functional limitations can be mitigated, reduced and/or controlled through exercise-based cardiac rehabilitation programs (EB-CRP) (32,33) in both ACS and HF (34-36).

A key variable of EB-CRP is to focus on reversing muscle dysfunction. The complexity of this essential and extensive anatomical tissue designed to accomplish roles in contraction, force generation, and movement turn it into a crucial element for exercise training (ET) and adaptation. Moreover, skeletal muscles have vital glucose metabolism and thermoregulation functions and were recently discovered as an endocrine organ with anti-inflammatory properties. To understand its importance, muscle tissue should not be considered as a simply biomechanical device that generates movement. Muscles must be understood as a complex machin with various interacting components, including the autonomic nerves for impulse transmission, vasculature for efficient oxygenation, and embedded regulatory and metabolic machinery for maintaining cellular homeostasis (37). These crucial functions and muscle physiology are impaired in CVD.

1.5 Skeletal muscle function and properties

Skeletal muscle is the most abundant tissue in the body. Although recent studies show that muscles have a crucial secretory functionality linked with essential biomarkers such as myokines and cytokines, their ability to contract and produce movement continues to be the primary functions of peripheral and respiratory muscles (38–40).

The functionality of muscles for generating force and movement depends on multiple domains and factors (Figure 3). Some of the most important properties of this tissue are electrical excitability, which means that muscles can respond to different stimuli generaed by electrical signals. Contractibility is the possibility to contract and create tension or force of contraction. Finally, extensibility, the ability to be stretched without suffering injury, and elasticity, the property allowing to recover original length and shape (41,42).

1.5.1 Skeletal muscle structure and physiology

The architecture of skeletal muscle is characterized by a very particular and well-described assembly and arrangement of thousands of muscle fibers that function as a unit with its associated connective tissue. Each muscle is wrapped in connective tissue (epimysium) (Figure 4), and each muscle fiber, in turn, is grouped into fascicles, surrounded by a sheath of connective tissue called the perimysium. At the same time, muscle fibers, nerves, and blood capillaries associated with it are enveloped by the endomysium. In skeletal muscles, these three layers of primary connective tissue or extracellular matrix that make up the muscle extend beyond the muscle fibers and form the tendon, the macroscopic force transmitter of the motor unit (Figure 4).

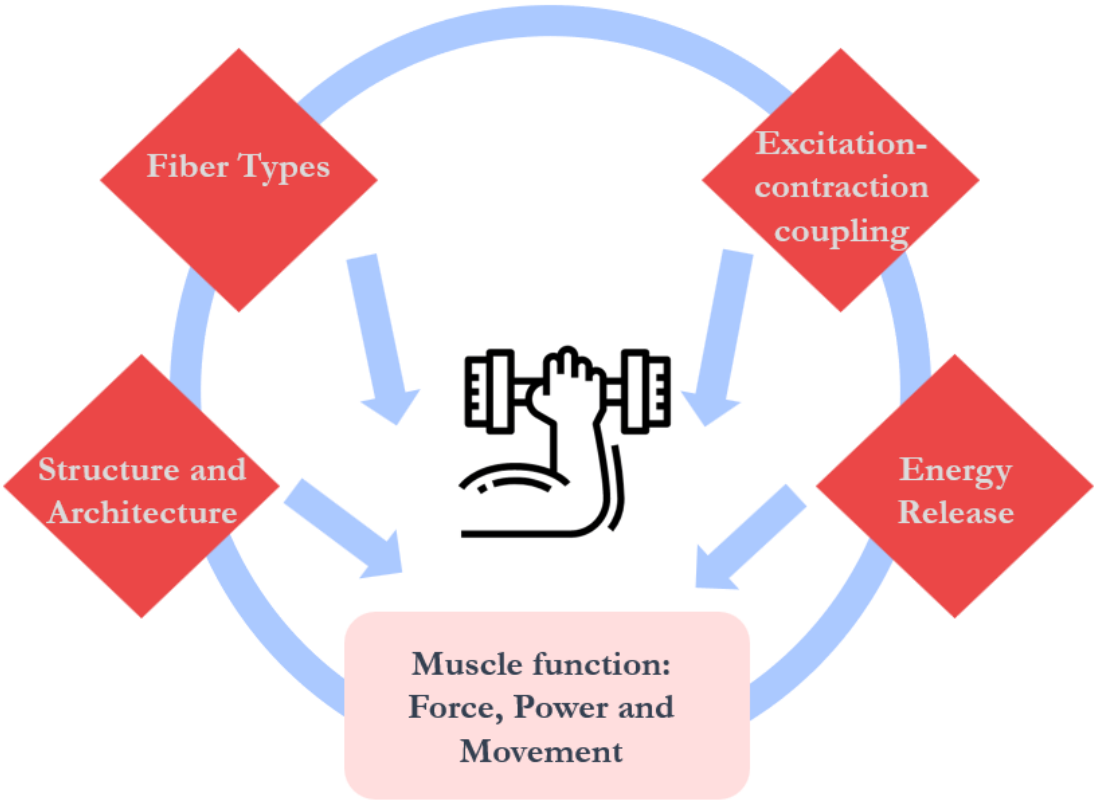

Figure 3. Interacting domains of skeletal muscle action. Adapted from Frontera & Ochala, 2015 (42).

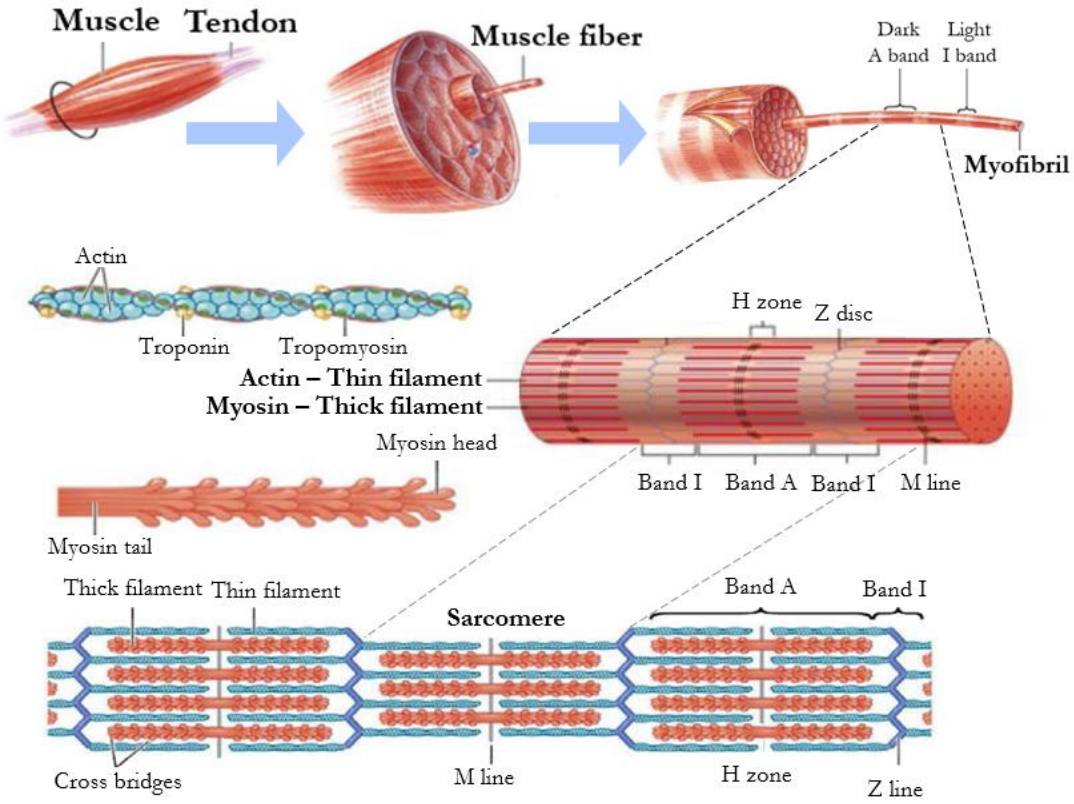

Figure 4. Structure of skeletal muscle: from whole muscle to individual fibers. Adapted from Frontera & Ochala, 2015 (42).

Physiologically, the force generated by a muscle depends on many factors, including the activation by the nervous system, its architecture (and the angle at which muscle fibers insert into the tendon known as the pennate angle), the muscle size, the space between myofilaments, the number of actin-myosin cross-bridges formed, the force generated by each cross-bridge, and the quality of the interaction between the cellular elements.

At the microscopical anatomy level, fibers are surrounded by a cell membrane or sarcolemma. Associated with the sarcolemma is a complex of several proteins physically connected to the internal myofilament structure, particularly to the actin protein in the thin filament (Figure 4). For these microfilaments to interact, adenosine triphosphate (ATP) is made available to an existing actin-myosin cross-bridge, and ATP binds to its attachment site on the myosin head. The ATPase, also in the myosin head, hydrolyzes the ATP resulting in adenosine diphosphate (ADP), phosphate (Pi), and the detachment of the existing cross-bridge (Figure 5). The muscle contraction cycle begins with the release of Ca^{2+} from the sarcoplasmic reticulum into the sarcoplasm. Once released, the Ca^{2+} ion binds to troponin, causing the displacement of tropomyosin, leaving the binding points of actin with myosin free and allowing them to join. At this point, the contraction cycle starts (42). The velocity, resistance, and metabolism will be determined by fiber type.

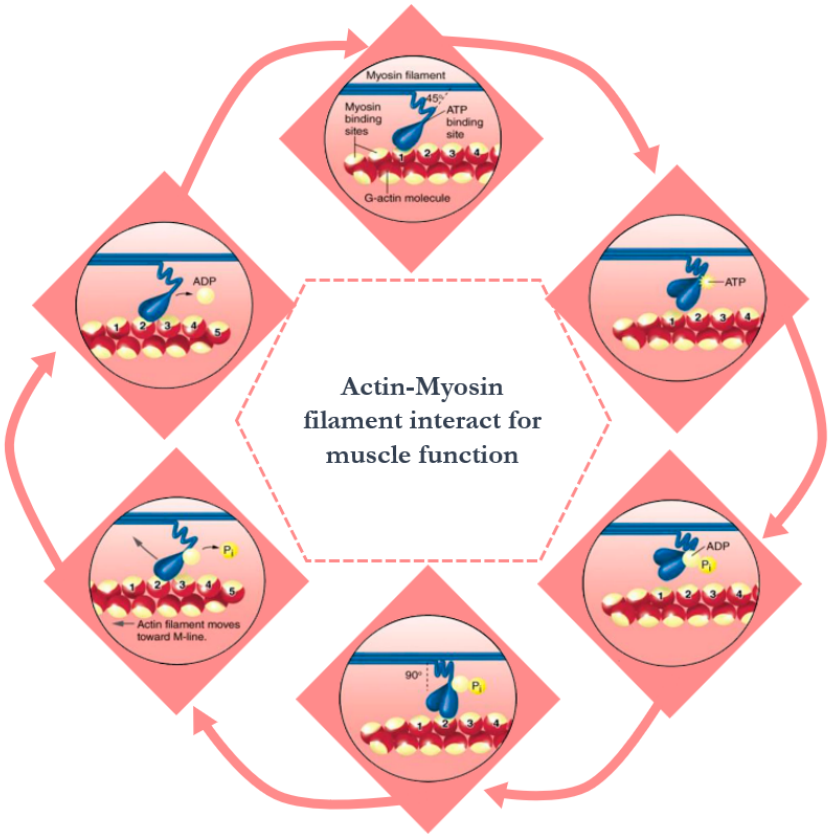

Figure 5. Sequence of events leading to the muscle contraction and force generation. Adapted from Frontera & Ochala, 2015 (42).

1.5.2 Skeletal muscle fiber type

Skeletal muscle tissue is heterogenous with different characteristics depending on the body's needs. Muscle fibers are the most prominent cells in the body and the main structural element of skeletal muscle morphology. There are at least four types of muscle fibers, characterized by distinct metabolism and function, type I, type IIa, type IIb, and type IIx. These respond to a large spectrum of functions, from low-intensity movements (posture, breathing), to fast and maximal contraction movements (sprinting, jumping) (43,44).

Different fiber types have at least three physiological characteristics: metabolism, contraction speed, and fatigue resistance. Additionally, the different fiber types exhibit different enzymatic activity. For instance, type IIa and type IIb fibers have high levels of glycolytic enzymes (low levels of mitochondria, highly developed sarcoplasmic reticulum), and type-I fibers have fatty-acid oxidative enzymes which means high levels of mitochondria and lesser developed sarcoplasmic reticulum. Furthermore, some muscle fibers are fast-twitch and oxidative thereby resisting fatigue better than others that are fast-twitch but glycolytic, thus more prone to fast fatigue. Fiber type composition, and thus metabolism, is a crucial element in muscles that must continuously generate contraction to maintain vital functions (43,44).

Muscle and fiber type distributions vary among multiple factors and species. When studying muscle physiology, looking at different fiber types is fundamental to understand muscular performance and efficiency. Based on some structural and functional characteristics, mammalian muscle fibers have been classified in detail (Table 1).

Table 1. Types and characteristics of the different fiber types. Adapted from Lopez Chicharro, 2008 (45).

Fiber type / **Characteristic**	**SO** *Slow oxidative*	**FOG** *Fast oxidative-glycolytic*	**FIG** *Fast Intermediate-glycolytic*	**FG** *Fast glycolytic*
Myosin heavy chain (MyHC) isoform	MyHC-I	MyHC-IIa	MyHC-IId/x	MyHC-IIb
Twitch time course	Slow	Moderately fast	Fast	Very fast
Power produced	Low	Medium	High	Very high
Fatigue resistance	High	Moderately high	Low	Very low
Endurance	Hours	<30 min	< few min	<1 min
Myosin ATPase activity	Low	Moderately high	High	Very high
Oxidative capacity	High	High	Low	Very low
Glycogen content	Low	High	High	High
Myoglobin content	High	Medium to high	Medium to high	Low
Mitochondrial density	High	Medium to high	Medium to high	Low
Capillary density	High	Medium to high	Medium to high	Low
Metabolic activity	Aerobic	Aerobic	Short term aerobic	Mainly anaerobic

SO = slow oxidative; FOG = fast oxidative-glycolytic; FIG = fast intermediate-glycolytic; FG = fast glycolytic; MHC = myosin heavy chain.

1.6 Skeletal muscle dysfunction in CVD

Muscle dysfunction has been investigated in different animal models of cardiac disease. Less is known, however, about muscle dysfunction in humans (46–49). In animal studies, an increase of oxidative stress with oxidation in contractile and energetic proteins produced changes in glycogen synthesis, calcium sensitivity, citrate synthase activity, and alterations of enzymes involved in mitochondrial energy production that contributes to diaphragm weakness and exercise capacity in HF (50–59).

1.6.1 Animal models of CVD

During the past four decades, basic and translational scientists have used animal models to investigate the pathophysiology of CVD, to improve disease prevention and treatment, and to evaluate new therapeutic strategies (60). Although animal models never completely resemble the clinical situation, they allow for obtaining direct information about specific events, offering reasonable control over several variables while applying accurate and typically invasive procedures that are difficult to employ in clinical studies (61).

1.6.2 Advantages and limitations of animal models of CVD

The main advantages of these models are using control groups and setting the conditions that could modify the results after varying one or more factors in a well-defined way (Figure 6). However, some limitations stem from the differences between human and experimentally induced disease, such as differences in genetic regulatory mechanisms or in factors that influence cardiovascular function (Figure 6). Moreover, there may be anatomical differences between phylogenetically distinct species, they may respond to different pathophysiological mechanisms, and also pharmacological treatments may act differently. For these reasons, the extrapolation of basic-experimental research findings to humans should always be carefully undertaken. It becomes essential to check which model to choose for each specific research objective (61,62).

Finding potential solutions to alterations induced by CVD will require to clearly understand the underlying causes and the experimental confirmation of putative treatments, both preventative and ameliorative. Each species and animal model has advantages and disadvantages. The choice of one or another model should be clearly decided to optimize the experimental design. Appropriate animal models are essential in this research, and a range of candidate models in different species have been developed (Figure 6) (49,63).

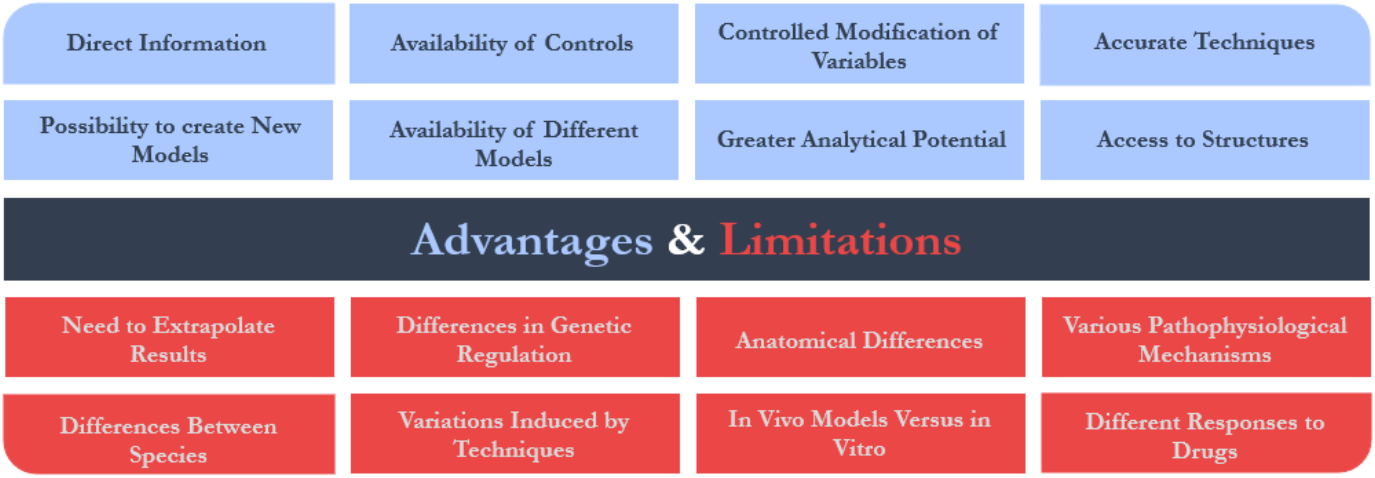

Figure 6. Main advantages and limitations of animal models used in research on cardiovascular disease. Adapted from Chorro and cols, 2009 (61).

1.6.3 Rodent models of CVD and skeletal muscle dysfunction

The most valuable of these models for experimental purposes is the small animal species/strains, especially the rodents. They have stronger reproductive ability and more clear feeding conditions with excellent maneuverability and detection ability of physiological indicators. Rodents have been widely used for the study of cardiovascular diseases. While reports of new models continue, especially of genetically modified mice, many such models are not well-characterized, particularly regarding skeletal muscle dysfunction (64,65).

Experimental rodent models mimicking coronary atherosclerotic heart disease, hypertensive heart disease, vascular disease, rheumatic heart disease, pulmonary heart disease, myocarditis, congenital heart disease and HF have been developed for translational research. The progress and research development in skeletal muscle dysfunction is closely linked with HF as this human disease is characterized by skeletal muscle wasting with structural, metabolic and functional abnormalities in the muscle tissue. These abnormalities are induced by reduced physical activity and catabolism caused by metabolic and hormonal derangements.

Metabolic abnormalities particularly impact through abnormal energy metabolism, development of mitochondrial dysfunction, a transition of myofibers from type I to type II and finally, resulting in clinical consequences such as muscle atrophy and reduced muscular strength. These alterations lead to muscle dysfunction that plays a central role in decreased exercise capacity of these CVD patients (66).

1.6.4 Mouse models of HF

HF is a secondary disease of various cardiovascular diseases, such as ACS/CAD. Usually, HF presents with impaired left ventricular function and reduced or preserved ejection

fraction. The pathophysiological changes lead to a hypertrophic response of the left ventricle (LV), interfering with LV mechanics and relaxation capacity, producing diastolic dysfunction. Continuous alteration of chronic deterioration or ischemic injury induces cardiomyocyte death resulting in decreased contractile force and wall thickness, leading to systolic dysfunction where the LV has impaired filling. Increased myocardial stiffness and diminished LV contractility are the primary hallmarks of HF and an objective to replicate and verify for models aimed at mimicking HF (67–69).

Several mouse models have been generated as tools to decipher HF pathophysiology and to develop new treatment strategies. These models are typically based on genetic modifications or pharmacological/surgical approaches, which can in fact be combined. The pathogenesis of HF is multifactorial and over the last few decades, several mice animal models have significantly advanced our understanding of the pathogenesis of this disease and skeletal muscle alterations have been explored.

1.6.5 Mice models of HF and muscle dysfunction

Among the many technical procedures and models to reproduce HF are general volume overload, LV pressure overload, right ventricular (RV) pressure overload, ischemic injury, genetic modifications and toxic cardiomyopathy (Figure 7). These procedures lead to aortic/pulmonary artery constrictions or aortocaval fistula reproducing HF (67).

Rodent models for studying the pathophysiology of HF should exhibit the cardiac alterations that characterize this disease and realistically mimic the muscle dysfunction that leads to well-known diaphragmatic weakness observed in patients with HF (70–72).

1.6.6 Diaphragm muscle dysfunction and impact in CVD

The relevance of diaphragmatic dysfunction is closely linked with the pathophysiology of cardiorespiratory dysfunction in CVD. Diaphragm weakness is caused by contractile apparatus dysfunction and fiber atrophy. These conditions and fiber types shifts determine a slower shortening velocity of contraction. Furthermore, these muscle dysfunctions trigger cardiovascular and pulmonary pathophysiological responses, in which sympathetic nervous activity and aging also are involved (Figure 8). It is well known that rodent models of HF exhibit functional diaphragm weakness and contractile dysfunction in both rats and mice. While weakness of the diaphragm was observed in models of left coronary artery ligation, aortic constriction, aortic stenosis-fistula, in a toxicological model of monocrotaline administration and in a transgenic model, diaphragmatic dysfunction has not been described

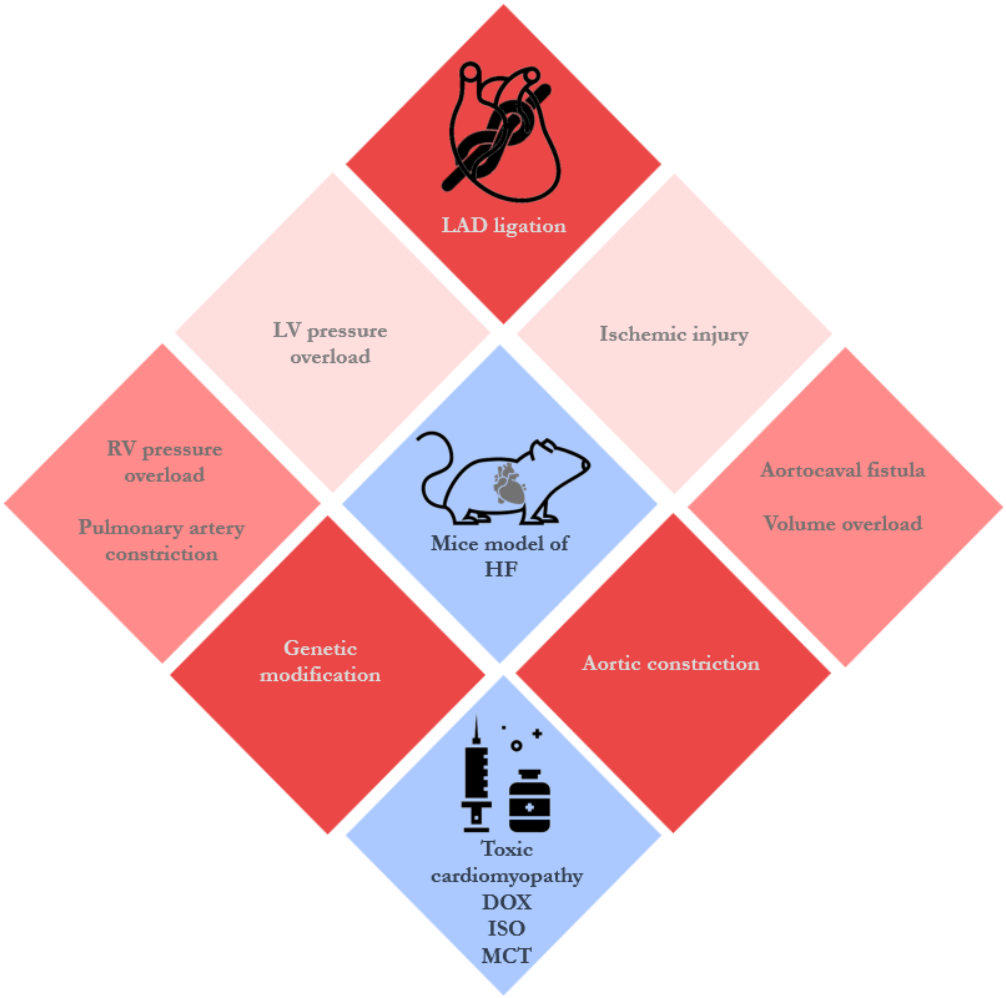

Figure 7. Schematic depicting selected strategies to induce heart failure in small animal models. DOX, doxorubicin; ISO, isoproterenol; LAD, left anterior descending artery; LV, left ventricular; MCT, monocrotaline; RV, right ventricular. Adapted from Rihele and cols, 2019 (67).

in any drug-induced mice model (50,52,53,58,74–76).

1.6.7 Diaphragmatic dysfunction in isoproterenol-induced HF model

Describing diaphragmatic dysfunction in some drug-induced HF mouse models is of interest. Indeed, contrary to surgeries to induce myocardial infarction or aortic ligation, drug-induced models are easy to implement, simply by placing a subcutaneous pump releasing isoproterenol (ISO). This model is helpful for expanding muscle dysfunction research and exploring potential therapeutic approaches.

Remarkably, there are no data on whether the ISO model of HF results in diaphragm dysfunction. An excessive dose of catecholamines, such as isoproterenol, produces diffuse myocardial destruction with cardiomyocyte necrosis and extensive fibrosis in animals and

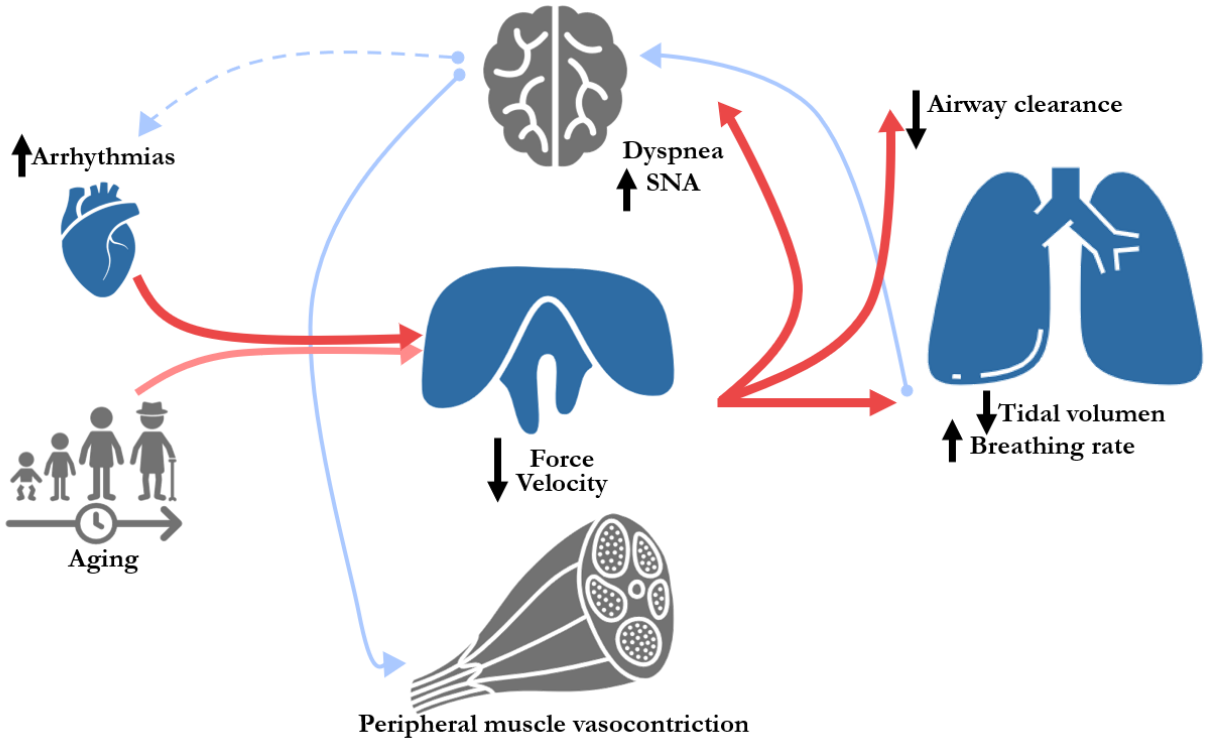

Figure 8. Relevance of diaphragm abnormalities to cardiovascular and respiratory pathophysiology in aging and heart failure. Adapted from Kelley and cols, 2017 (73).

CHF = chronic heart failure; SNA = autonomic nervous system.

humans. The mechanism underlying myocardial damage is likely related to an imbalance between oxygen supply versus demand due to myocardial hyperactivity. In mice, continuous infusion of isoproterenol has been shown to induce cardiac dysfunction (77–79).

However, indirect data from research where ISO is applied in different doses/modes strongly suggest that ISO induces an increase in the mechanical performance of the diaphragm, contrary to that observed in HF patients. Indeed, it was observed that ISO enhanced contractility of the diaphragm in canine, rat and mice model of aorta constriction. Therefore, diaphragmatic muscular behavior in a ISO-induced HF mice model is unknown and not clear (75,80,81).

1.7 Translational impact of muscle dysfunction

The patient's prognosis and systemic repercussions of muscle dysfunction and exercise intolerance are more explored in secondary prevention. Patients with CVD usually have skeletal muscle abnormalities, such as reduced muscle fiber type I, decreased number of capillaries per muscle fiber and mitochondrial dysfunction, which all contribute to reduced cardiorespiratory capacity (82).

Muscle weakness frequently observed in respiratory and peripheral muscles is strongly associated with the worst prognosis. Nevertheless, changes in skeletal muscle are still sparsely available and more studies in this CVD population are clearly warranted. As previously commented, most of these muscular complications and prognosis variables are mitigated, reduced and/or controlled through EB-CRP (32,33).

1.7.1 Exercise-based Cardiac rehabilitation program

Historically, CVD patients were assumed to be at risk to exercise and were commonly discouraged from participating in physical activity. Contrary to these concerns, the safety and benefits of exercise and physical activity in the CVD population it has been strongly observed in many studies. Accordingly, it is currently accepted by clinicians and scientists that exercise can be a therapeutic tool (83).

EB-CRP is defined as "an interdisciplinary program that includes exercise training, cardiac risk factor modification, psychosocial and outcomes assessment". EB-CRP is mainly based on the benefits and favorable effects of physical exercise. However, comprehensive interdisciplinary rehabilitation programs also focus on optimized pharmacotherapy, psychological support, lifestyle modification, educational sessions focusing on risk factors, among others (Figure 9) (84–86).

1.7.2 EB-CRP efficacy and beneficial effects

Among the benefits of completion and participation in EB-CRP are improvement in exercise tolerance and cardiorespiratory capacity, optimization and control of risk factors, regulation of lipoprotein profile, control of body weight, balance of blood glucose and blood pressure levels, favor smoking cessation, lessening depression and anxiety, improve in QoL, decrease hospital readmission and morbimortality rates (5,35,87–91). These benefits have been demonstrated in various cardiac diseases: IHD, ACS, HF, heart transplantation, cardiac arrhythmias and severe arterial hypertension (92). Several meta-analyses have demonstrated efficacy, effectiveness and cost-effectiveness.

In summary, the practice of EB-CRP as an essential health intervention is advocated at the international and national level as necessary secondary prevention not only to prevent but also to delay or alleviate CVD (87,93–97). However, despite outcome benefits, positive economic evaluation and 1A recommendation in national (Spanish Society of Cardiology) and international guidelines (European Society of Cardiology, American Heart Association and American College of Cardiology), EB-CRP, remains underused.

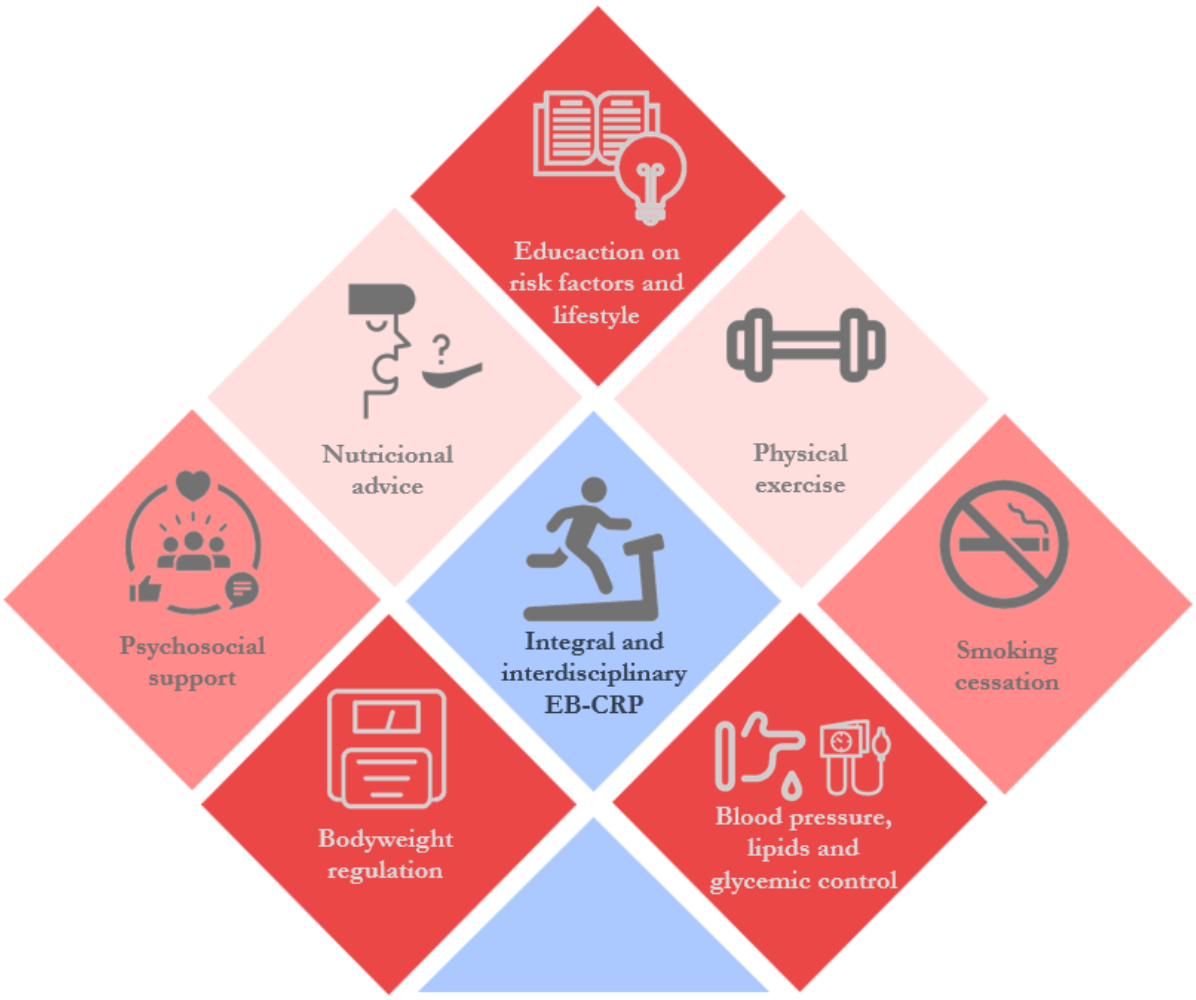

Figure 9. Core components of comprehensive-interdisciplinary exercised-based cardiac rehabilitation program. Adapted from Winnige and cols, 2021 (86).

1.7.3 EB-CRP participation

As some studies have shown, participation in this type of programs continues to be lower than expected. Despite variability in participation rates between different countries, more than half of eligible patients do not attend EB-CRP. While in other countries of Europe participation ranges are 49%-65%, higher rates than UK or USA, in Spain a few years ago was considered that even were few interdisciplinary EB-CRP units and that these were usually performed in a hospital setting with participation near to 50%. Studies have compared EB-CRP participants with those who do not participate, ultimately showing that they have different characteristics (48,98–101).

Increasing participation rates continues to be a strong current challenge in the EB-CRP setting including an evolution to incorporate "younger" or "middle-aged" CVD patients and their needs/preferences. To provide optimal and integrative health care, it is critical to know why patients choose not to participate in EB-CRP. This knowledge would improve

participation, adherence and prognosis, involving cardiac rehabilitation process closer to well-known patient-centered care model (102).

1.7.4 Impact of not complete EB-CRP

Patients who do not complete EB-CRP or delay starting it have a worst prognosis than those who complete rehabilitation. In both cardiorespiratory and cardiac morbimortality outcomes. Studies defined incomplete participation in different ways. Some consider as incomplete participation assistance <75% of sessions. Others defined drop-out as attending 50% of these sessions or less, which for some consensus, would be a cohort of 23 sessions (101,103–105).

Remarkably, most of these studies analyze prognosis in patients who drop-out or were never referred to EB-CRP. There are no data about the impact of not participating in exercise/physical component in patients adhering to other core-components such as nutritional advice, psychological support, smoking cessation and education sessions. No studies have been published exploring whether initial attitude can affect the prognostic impact of non-attendance or drop-out EB-CRP patients even when they initially accepted participation.

1.7.5 Routinary stratification tools for exercise settings, participation and adherence

In addition to the above-mentioned well-established modalities of EB-CRP in a hospital setting, research has also focused on other feasible training alternatives for improving participation and adherence, including those for global pandemic situation: community-based, home-based and remotely-monitoring training. The goal is to set up a selection of options and adapt the EB-CRP to the patient's needs, experience, preferences and risk profile (86).

Cardiac risk stratification (RS) becomes critical to obtain the beneficial effects of EB-CRP and ensure safety during physical exercises. RS is essential for correctly prescribing physical exercise and selecting the correct setting, modality, supervision and monitoring during exercise practice (106,107). The main advantage of patient's initial evaluation of EB-CRP is that by performing a stress test, patients can be stratified by risk. This stratification helps decide where the patients will perform the exercise training, but, surprisingly, it is unknown whether this classification can help identify patients with a bad prognosis.

1.7.6 Easy-to-calculate risk stratification and prognosis

The Spanish Society of Cardiology (SSC) developed one of the easy-to-calculate RS protocols, stratifying patients based on several parameters (Table 2). Parameters are obtained mainly from cardiorespiratory stress test and echocardiogram during hospitalization or after hospital discharge and patients are categorized as low, mid, and high risk according to this classification. However, the SSC-EXCELENTE cardiac rehabilitation committee later proposed classifying patients only as low (L) or no-low risk (NL). This classification is consistent with other international entities and studies that indicate that all patients who are not classifiable as low risk should be considered high risk (107,108). As mentioned before, even though almost all patients could be easily stratified, it is unknown whether this simple classification can help identify patients with the worst prognosis. Due to the routine nature of this RS, if it has prognostic utility, it could be useful for clinicians for being fast and easy-to-calculate.

Table 2. Risk stratification by Spanish Society of Cardiology. Adapted from Velasco and cols, 2000 (108).

Bajo riesgo	*Riesgo medio*	*Alto riesgo*
Curso hospitalario sin complicaciones Ausencia de isquemia Capacidad funcional > 7 METS	Aparición de angina Defectos reversibles con talio de esfuerzo Capacidad funcional entre 5-7 METS	Reinfarto. ICC hospitalaria Depresión de ST> 2mm con FC<135 lat/min Capacidad funcional <5 METS con o sin depresión de ST
FE > 50%	FE del 35-49%	FE < 35%
Ausencia de arritmias ventriculares severas		Respuesta hipotensiva al esfuerzo Arritmias ventriculares malignas

ICC: Insuficiencia cardíaca congestiva.

1.7.7 Easy-to-calculate risk stratification variation with exercise compliance

Finally, completing the exercise component of EB-CRP is associated with an improvement in cardiorespiratory capacity measured by an increase in the metabolic equivalent (METS) achieved in the exercise stress test and has also been associated with an increase in LVEF, both important variables in RS process (109,110). However, whether with improvement can lead to change this RS and prognosis is largely unknown.

Chapter II:
HYPOTHESIS AND AIMS OF THE THESIS

From a translational perspective: to characterize the muscular function in the diaphragm of an isoproterenol-induced heart failure mice model.

From a clinical perspective: to compare clinical outcomes and prognosis utility of RS in different EB-CRP participation levels and exercise compliance.

2 Hypothesis of the Thesis

The isoproterenol-induced heart failure mice model potentially increases diaphragm contractility.

Patients who do not-attend EB-CRP potentially have a bad prognosis regardless initial attitude.

Easy-to-calculate and routinary risk stratification protocol of the Spanish Society of Cardiology could potentially be useful for identifying patients with bad prognosis, and exercise participation will change stratification.

2.1 Aims

1. To describe the changes in the muscular function of an isoproterenol-induced heart failure mice model.

i. To assess structural and functional changes of diaphragm muscle in an isoproterenol-induced heart failure mouse model.

ii. To compare diaphragm muscle function and structure with healthy controls.

2. To analyze the impact of an EB-CRP in patients after acute coronary syndrome.

iii. To identify prognostic differences between different exercise compliance.

iv. To differentiate reasons for not performing exercise component.

3. To test an easy-to-calculate risk score based on routinary outcomes of patients attending EB-CRP.

v. To analyze the prognostic utility of risk stratification protocol.

vi. To assess if exercise training can modify stratification and improve prognosis.

Chapter III:
Articles in this thesis

Three of the total scientific articles produced during Ph.D student activities are included in the core of this Thesis. The Ph.D student was the first author in all of them. Here they are listed regarding the Aims of the Thesis:

Aims i and ii: *To describe the changes in the muscular function of an isoproterenol-induced heart failure mouse model. To assess diaphragm muscle functional and structural changes in this model and to compare function and structure with healthy controls.*

- ✓ **I. Cabrera-Aguilera**, B. Falcones, A. Calvo-Fernández, B. Benito, E. Barreiro, J. Gea, R. Farré, I. Almendros and N. Farré, "The conventional isoproterenol-induced heart failure model does not consistently mimic the diaphragmatic dysfunction observed in patients". PLoS One., vol. 15, pp. 1–17,. Jul 2020.
    - IF = 3.24, Q2/T2 (26/72)

Aim iii and iv: *To analyze the impact of an EB-CRP in patients after acute coronary syndrome. To identify prognostic differences between different exercise-compliance and to differentiate reasons for not performing exercise training.*

- ✓ **I. Cabrera-Aguilera**, C. Ivern, N. Badosa, E. Marco, L. Salas-Medina, D. Mojón, M. Vicente, M. Llagostera, N. Farré and S. Ruiz-Bustillo, "Impact of and Reasons for Not Performing Exercise Training After an Acute Coronary Syndrome in the Setting of an Interdisciplinary Cardiac Rehabilitation Program: Results From a Risk-Op-Acute Coronary Syndrome Ambispective Registry". Front. Physiol., vol. 12, pp. 1–8, Nov. 2021.
    - IF = 4.755, Q1/T1 (20/81)

Aims v and vi: *To test an easy-to-calculate risk score based on routinary outcomes of patients attending EB-CRP. To analyze the prognostic utility of risk stratification protocol and to assess if exercise training can modify stratification and improve prognosis.*

- ✓ **I. Cabrera-Aguilera**, C. Ivern, N. Badosa, E. Marco, X. Duran, D. Mojón, M. Vicente, M. Llagostera, N. Farré and S. Ruiz-Bustillo, "Prognostic Utility of a New Risk Stratification Protocol for Secondary Prevention in Patients Attending Cardiac Rehabilitation". J. Clin. Med., vol. 11, pp. 1910–21,. Mar 2022.
    - IF = 4.964, Q2/T1 (54/172)

Chapter IV:
SCIENTIFIC ARTICLE I

The conventional isoproterenol-induced heart failure model does not consistently mimic the diaphragmatic dysfunction observed in patients

I. Cabrera-Aguilera, B. Falcones, A. Calvo-Fernández, B. Benito, E. Barreiro, J. Gea, R. Farré, I. Almendros and N. Farré.

PLoS One., vol. 15, pp. 1–17, Jul 2020.

IF = 3.24, Q2/T2 (26/72)

PLOS ONE

RESEARCH ARTICLE

The conventional isoproterenol-induced heart failure model does not consistently mimic the diaphragmatic dysfunction observed in patients

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Citation: Cabrera-Aguilera I, Falcones B, Calvo-Fernández A, Benito B, Barreiro E, Gea J, et al. (2020) The conventional isoproterenol-induced heart failure model does not consistently mimic the diaphragmatic dysfunction observed in patients. PLoS ONE 15(7): e0236923. https://doi.org/10.1371/journal.pone.0236923

Editor: Vincenzo Lionetti, Scuola Superiore Sant'Anna, ITALY

Received: May 20, 2020

Accepted: July 16, 2020

Published: July 30, 2020

Data Availability Statement: All relevant data are within the manuscript.

Funding: ICA is supported by ANID-PFCHA – Chilean Doctorate Fellowship 2017; Grant No. 72180089. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Heart failure (HF) impairs diaphragm function. Animal models realistically mimicking HF should feature both the cardiac alterations and the diaphragmatic dysfunction characterizing this disease. The isoproterenol-induced HF model is widely used, but whether it presents diaphragmatic dysfunction is unknown. However, indirect data from research in other fields suggest that isoproterenol could increase diaphragm function. The aim of this study was to test the hypothesis that the widespread rodent model of isoproterenol-induced HF results in increased diaphragmatic contractility. Forty C57BL/6J male mice were randomized into 2 groups: HF and healthy controls. After 30 days of isoproterenol infusion to establish HF, *in vivo* diaphragmatic excursion and *ex vivo* isolated diaphragm contractibility were measured. As compared with healthy controls, mice with isoproterenol-induced HF showed the expected changes in structural and functional echocardiographic parameters and lung edema. isoproterenol-induced HF increased in vivo diaphragm excursion (by ≈30%, $p<0.01$) and increased by ≈50% both *ex vivo* peak specific force ($p<0.05$) and tetanic force ($p<0.05$) at almost all 10–100 Hz frequencies ($p<0.05$), with reduced fatigue resistance ($p<0.01$) when compared with healthy controls. Expression of myosin genes encoding the main muscle fiber types revealed that *Myh4* was higher in isoproterenol-induced HF than in healthy controls ($p<0.05$), suggesting greater distribution of type IIb fibers. These results show that the conventional isoproterenol-induced HF model increases diaphragm contraction, a finding contrary to what is observed in patients with HF. Therefore, this specific model

seems limited for translational an integrative HF research, especially when cardio-respiratory interactions are investigated.

Introduction

Heart failure (HF) is a very prevalent disease and a major public health problem with considerably associated mortality and health system expenditure. Noteworthy, the current high prevalence of HF is expected to increase worldwide as a result of the obesity epidemics and population ageing [1, 2]. In addition to primary cardiocirculatory alterations, HF also impacts the respiratory system by inducing lung edema and breathlessness/dyspnea [3, 4], particularly in patients with advanced stage of the disease and appearing either in stable conditions and during acute HF exacerbations. Remarkably, it has been reported that patients with HF also present weakness of the respiratory muscles, specifically the diaphragm [5], which in association with lung edema could contribute to hinder correct ventilation, thus promoting hypoxia and dyspnea.

Accordingly, animal models for optimally studying the pathophysiology of HF not only must exhibit the cardiac alterations characterizing the disease but also should realistically mimic the diaphragmatic dysfunction observed in patients with HF, particularly when studying exercise interventions in HF animal models [6–8]. The HF model based on infusion of isoproterenol is widely used since it features realistic structural and functional cardiac alterations and has the advantage of being experimentally simple [9]. Indeed, contrary to requiring major surgeries as when HF is induced by myocardial infarction or aortic ligation, HF is induced almost non-invasively by simply placing a subcutaneous pump to continuously infuse isoproterenol. It is noteworthy, however, that there are no data describing whether this widely used HF model results in diaphragm dysfunction. Nevertheless, indirect data from research in other fields where isoproterenol is applied at different doses and modes suggest that this agent elicits an increase in the contractile performance of skeletal muscles [10, 11] and in particular of the diaphragm. For instance, it was observed that intravenous injection of isoproterenol enhanced contractility of canine diaphragm [12]. Moreover, in isolated muscle testing of rats with septic peritonitis, isoproterenol added to organ bath increased diaphragmatic contractility [13], similarly as reported when imposing cardiac pressure overload by transverse aorta constriction in a rodent model [14].

In case that, as indirectly suggested from the afore mentioned studies [12–14], application of systemic isoproterenol with the specific dosage and duration as in the HF model would result in diaphragm reinforcement —exactly the reverse alteration found in HF patients— the cardio-respiratory interest of this model would be challenged. Therefore, the aim if this study was to test the hypothesis that the widespread rodent model of HF based on isoproterenol infusion results in increased diaphragmatic contractility. To this end, diaphragmatic function in the conventional murine model of isoproterenol-induced HF was assessed *in vivo* by ultrasound echography and *ex vivo* by isolated muscle contractility testing.

Materials and methods

Animals

The study was carried out on forty adult male C57BL/6J mice (10 weeks old; Charles River Laboratories, Saint Germain sur L'arbresle, France) maintained on a 12 h light/dark cycle room (light on 8:00 am to 8:00 pm) with water and food *ad libitum*. In a first series, 20 mice

were randomly assigned to HF and healthy controls (N = 10 each) and were subjected to non-invasive evaluation of muscle function with echocardiography. Similarly, in a second series of mice, *ex-vivo* diaphragm contraction was measured. Diaphragm samples were used for assessing gene expression of myosin types. The intervention protocols were approved by the Ethics Committee for Animal Experimentation of the University of Barcelona.

Heart failure model

HF was induced by continuous infusion of isoproterenol with an osmotic pump, following the conventional procedure in this model [9]. Briefly, mice were anesthetized with a mixture of inhaled isoflurane and oxygen-enriched air (1.25% during induction and 1% during maintenance) and a small incision was made on the back of each animal between the shoulder blades after removing the hair from the area using depilatory cream. The skin was carefully separated from underlying connective tissues using blunt-ended scissors and an osmotic mini-pump (Alzet, model 1004) containing isoproterenol (Sigma Aldrich) at 30 mg/kg per day dissolved in sterile 0.9% NaCl solution or only 0.9% NaCl solution (for healthy controls) was implanted subcutaneously for delivery pharmacological agent or placebo for 30 days and the incision was sutured with surgical staples (Autoclip, Fine Science Tools). The procedure was performed under aseptic conditions. The surgery platform was continuously warmed to maintain body temperature until the end of anesthesia. Buprenorphine (0.1 mg/kg) was subcutaneously administered 10 minutes before surgery and after 24 hours. Suture staples were removed 7 days after surgery.

Assessment of heart failure by echocardiography

At baseline (before pump implantation) and 30 days after treatment with isoproterenol or saline (end-point), echocardiography (Vivid IQ and L8-18i-D Linear Array 5-15MHz, General Electric Healthcare, Horten, Norway) was measured by a single operator (NF) who was blind to the animal group following a standard protocol [15]. Briefly, using the same anesthesia as for mini-pump implantation, chest and abdominal hair were removed using depilatory cream, then the mouse was placed in supine position on a continuously warmed platform to maintain body temperature and the four limbs were fixed. Ultrasound gel was applied on the left hemithorax and the following echocardiographic indices were subsequently computed: left ventricular end-diastolic (LVEDD) and end-systolic diameter (LVESD), left ventricular ejection fraction (LVEF) and fraction shortening (FS).

In vivo diaphragmatic echography

Diaphragmatic echography was performed immediately after echocardiography by the same operator and with the same device following a standard protocol for non-invasively measuring diaphragm function in mice [16]. After applying gel on the area overlying the diaphragm just below the rib cage, an ultrasound probe (Vivid IQ and L8-18i-D Linear Array 5-15MHz, General Electric Healthcare, Horten, Norway) was placed along the transverse mid-sternal axis of the mouse, in order to locate the diaphragm on both sides of the body and M-mode was used to measure the diaphragm movement during normal breathing cycles, detecting contraction (positive deflection) and relaxed state (negative deflection) of diaphragm. Diaphragmatic excursion was quantified as the amplitude of movement between the lowest and peak point of the contraction.

Ex vivo assessment of diaphragmatic contractile function

Contractile function of the diaphragm at end point was assessed *ex vivo* using an isolated muscle test system (Aurora Scientific, Aurora, ON, Canada). All force data were recorded using a customized software implemented in LabVIEW (National Instruments, Austin, TX, USA) at a sampling rate of 1000 Hz and analyzed with MATLAB (The MathWorks, Natick, MA, United States). Diaphragm dissection and preparation of muscle strips were carried out following previously described procedures [17]. Immediately after euthanasia by exsanguination, diaphragms were dissected with ribs attached and placed into ice-cold buffer (118 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L KH_2PO_4, 0.57 mmol/L $MgSO_4$, 25 mmol/ L HEPES and 5.5 mmol/L glucose; pH 7.2). This ringer solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2. Diaphragm strips were prepared with the ribs at the distal end and the central tendon at the proximal end. The side of the rib was anchored and kept fixed, the central tendon was attached to a force transducer (305C Dual-Mode Muscle Lever, Aurora Scientific) using sutures [18] and the diaphragm strip was submerged into the above oxygenated ringer solution at 22˚C to prolong muscle stability for testing. The muscle strip remained at rest for 5 minutes prior to functional testing. Supramaximal stimulation conditions and optimum length was determined following established procedures [17].

A single twitch was elicited for 3 times (supra-maximal stimulation, 0.5 milliseconds) from which twitch force (peak force), time to peak force (contraction time) and time to 50% relaxation (half-relaxation time) were determined. The force-frequency relationship was then determined by sequentially stimulating the muscle strips at 10–100 Hz (10 Hz intervals) for 1 second at each stimulus frequency interspersed by 2-minutes recovery intervals between each stimulus, allowing measuring the maximum tetanic force. Fatigue resistance was measured as the decay time in force production while the diaphragm was stimulated continuously at 50 Hz for 40 seconds. From this curve we calculated the time until the initial maximum force decreased to 50% [18] and the strength decrement index (SDI) as a decay of force production at second 30 respect to the maximum force production [19]. Finally, the ribs and central tendon were removed, and the wet mass of the muscle tissue was weighted. Muscle cross-sectional area (CSA) was computed as CSA = m/(l·d), where m and l are strip mass and length, respectively, and d (= 1.06 g/cm^3) is muscle density [17, 20]. Preparation of diaphragm strips was performed by a researcher (IC-A) who was blind to the mice groups. Indeed, the optimum length of diaphragm strips did not show significant differences when comparing HF (7.74 ± 0.15 mm^2) and healthy control (7.62 ± 0.33 mm^2) mice (p = 0.751; t-test). Strip weight was 23.8 ± 1 mg and 23.5 ± 1 mg for HF and healthy controls, respectively, with no difference between groups (p = 0.866; t-test). Consistently, strip CSA showed no significant differences when comparing HF (3.0 ± 0.1 mm^2) and healthy controls (3.2 ± 0.2 mm^2) mice (t-test p = 0.604).

Myosin gene expression in the diaphragm

Diaphragm samples extracted from the animals immediately after sacrifice were snap frozen in liquid nitrogen and stored at -80˚C for further analysis. All reagents were purchased from ThermoScientific (Waltham, MO) unless specified. RNA was isolated using the RNeasy kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Briefly, after all diaphragm samples were collected, they were thawed and immediately lysed in a Polytron PT2100 homogenizer (Kinematica AG, Lucerne, Switzerland). RNA isolated from the tissue was employed to synthetize cDNA by reverse transcription polymerase chain reaction (PCR; TaqMan Reverse Transcription Reagents). Afterwards, changes in gene expression were analyzed by qPCR using the Taqman Fast Advanced Master Mix in a StepOne Plus thermocycler.

The candidate genes analyzed were chosen regarding its association with a specific muscle fiber type. Hence, the muscle fiber type MyHC-I correlates with *Myh6*, MyHC-IIa with *Myh2*, MyHC-IId/x with *Myh1* and MyHC-IIb with *Myh4* [21, 22]. Expression of the four genes was normalized to the expression of peptidylprolyl isomerase A (*PPIA*) used as an internal control. Relative gene expression levels are expressed as fold-change of the $2^{-\Delta Ct}$ compared to the baseline group of healthy control mice [23].

Assessment of lung edema

Lungs obtained immediately after diaphragm excision were stored at -80 ˚C and subsequently thawed at room temperature for 4 h, dried in an oven at 80 ˚C for 48 h and weighted again. Edema in each lung was assess as the wet/dry (W/D) weight ratio [24].

Statistics

All data are presented as mean ± SEM. Comparison of *ex vivo* variables between HF and healthy control groups were carried out by t-tests. When normality tests failed, the Mann-Whitney non-parametric test was used. In the case of *in vivo* variables (echocardiography and diaphragm echography) where data for each animal were available both at base line and end-point, comparisons were carried out for the variable change from baseline to end-point. For all tests, $p<0.05$ was considered as statically significant.

Results

As expected from previous reports using the isoproterenol model, mice showed echocardiographic indices characteristic of HF. As a result of random distribution of animals among HF and healthy control groups, baseline cardiac indices (LVEDD = 3.49 ± 0.05 mm, LVESD = 2.27 ± 0.06 mm, LVEF = 71.20 ± 1.29%, FS = 35.20 ± 1.08%) did not show significant differences between groups. However, at end-point mice in the HF group showed a significant increase in structural parameters (LVEDD and LVESD) and a significant decrease in functional parameters (LVEF and FS) as compared with healthy controls, confirming heart hypertrophy and decay in cardiac function (Table 1). As indicated in Table 2, HF mice showed slight but significant increase in body weight as compared with healthy controls (Table 2). Moreover, organ weight showed that the animals in HF group exhibited heart hypertrophy (Table 2). Consistently with HF, mice in the isoproterenol group had pulmonary edema since their lung W/D weight ratio was significantly greater than in healthy controls (Table 2).

In contrast with findings in HF patients, diaphragm function in mice subjected to isoproterenol-induced HF was considerably enhanced as compared with healthy animals, both in *in vivo* and *ex vivo* measurements. *In vivo* echography confirmed that, whereas diaphragmatic excursion at baseline was 2.12 ± 0.10 mm, with no significant differences between groups

Table 1. The isoproterenol-induced Heart Failure (HF) model significantly modifies echocardiographically-measured left ventricular structure and function.

	Baseline	Healthy ControlΔ	Heart FailureΔ	p value
LVESD (mm)	2.27 ± 0.06	-0.17 ± 0.10	-0.54 ± 0.05	0.008
LVEDD (mm)	3.49 ± 0.05	-0.13 ± 0.11	-0.59 ± 0.05	0.019
LVEF (%)	71.20 ± 1.29	-0.80 ± 1.75	-8.50 ± 1.37	0.003
FS (%)	35.20 ± 1.08	-0.70 ± 1.45	-6.20 ± 0.98	0.006

Δ indicates the change in heart variable from baseline to day 30 after isoproterenol or placebo infusion start for each heart failure and in healthy control animals, respectively (n = 10 each group). LVEED: end-diastolic diameter; LVEF: left ventricular ejection fraction; FS: fraction shortening. Values are mean ± SEM.

https://doi.org/10.1371/journal.pone.0236923.t001

Table 2. The isoproterenol-induced Heart Failure (HF) model induces cardiac hypertrophy and lung edema.

	Healthy Control	Heart Failure	p value
Body weight (BW) (g)	28.71±0.52	30.77±0.61	0.028
Heart weight (HW) (g)	0.15±0.004	0.19±0.012	0.0001
Normalized HW (100·HW/BW)	0.52±0.01	0.64±0.05	0.011
Lung edema (W/D)	4.58±0.19	5.93±0.32	0.004

Data were measured in the HF and heathy control and the groups at end point (after 30 days of continuous perfusion of isoproterenol or placebo, respectively). Lung edema index was measured as the ratio between wet (W) and dry (D) lungs (see Methods for explanation). Values are mean ± SEM.

https://doi.org/10.1371/journal.pone.0236923.t002

(p = 0.600), the increase in diaphragm excursion was significantly higher in HF mice as compared with healthy animals at the end of the experiment (Fig 1).

Moreover, *ex vivo* muscle assessment also showed increased diaphragm contractility in isoproterenol-induced HF as compared with healthy controls. Indeed, HF diaphragms exhibited a significant increase in peak specific twitch force in response to a single supra-maximal stimulus compared with healthy controls (Fig 2).

However, contraction time corresponding to this stimulus did not significantly (p = 0.397) differ between HF (0.03±0.03 ms) and healthy controls (0.03±0.04 ms). Also, non-significant differences (p = 0.294) were found in half relaxation time (0.03 ± 0.003 ms for healthy controls and 0.04 ± 0.003 ms for HF animals). Maximum force production observed with tetanic contraction using a continuous stimulus was higher in HF animals (Fig 3).

We also evaluated the tetanic contraction at increasing stimulation frequencies to describe the force-frequency relationship, confirming a significant increase of force production in almost all frequencies in the HF group when compared with healthy mice (Fig 4).

Finally, fatigue resistance experiments showed a significant decrease in time to half initial force in HF when compared with healthy control mice and a significant increase in the same group in the strength decrement index (SDI) at 30 seconds (Fig 5), indicating increased fatigue in HF diaphragms.

Gene expression of *Myh6*, *Myh2*, and *Myh1* at the diaphragm samples showed no significant differences when comparing HF and healthy mice. By contrast, *Myh4* expression showed a significant 2-fold increase in HF animals (Fig 6).

These results support a greater predominance of type IIb fibers over other types of muscle fibers in diaphragm samples of isoproterenol-induced HF animals.

Discussion

The results of this study reveal that the conventional HF rodent model based on continuous infusion of isoproterenol for 30 days is associated with considerable enhancement of diaphragm contractility. Therefore, whereas this model is very effective in mimicking the cardiac alterations and lung edema characterizing HF, it fails in reproducing the well-known weakening of diaphragm in patients with HF [5], which has been extensively documented by measuring muscle strength in voluntary maximal inspiratory or sniff maneuvers [24–31] as well as in no-volitional measures using phrenic nerve stimulation [25, 29, 32, 33], and which is associated with breathlessness/dyspnea, loss of functional capacity, exercise intolerance, reduced levels of quality of life and survival in HF patients [34–39].

The methodology of the present study for both setting the HF model and for assessing diaphragm contractibility has been widely used in the literature. Indeed, we implemented the mouse model of HF by applying a conventional procedure (subcutaneous pump, dose and

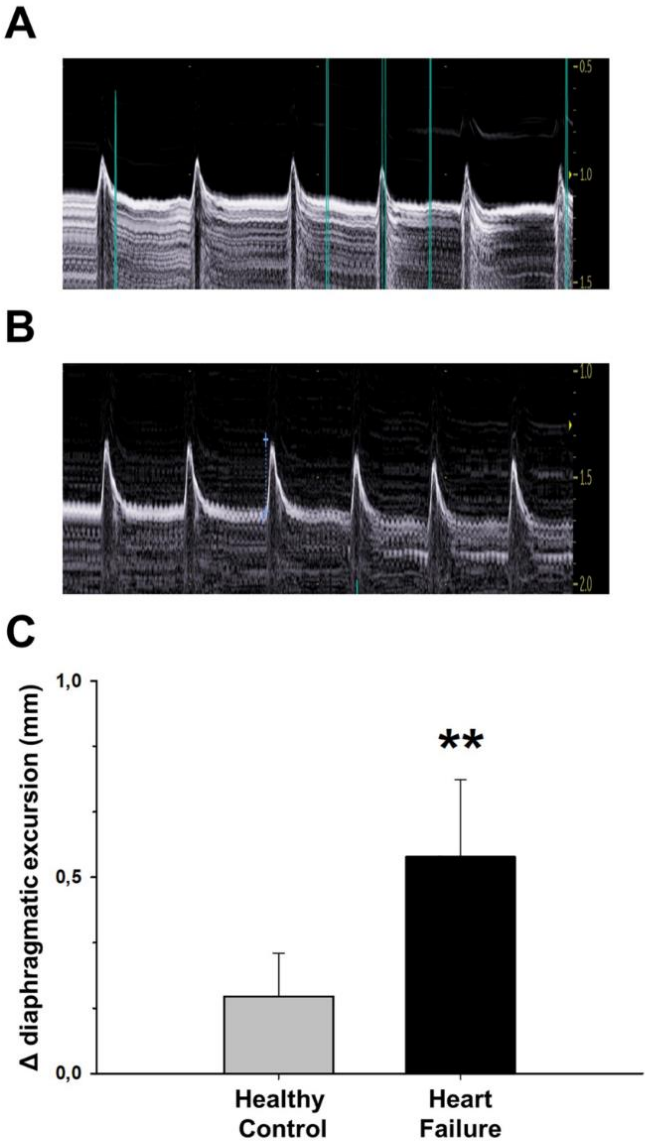

Fig 1. The isoproterenol-induced Heart Failure (HF) model significantly modifies echocardiographically-measured diaphragm function. Diaphragm echography in a representative HF mouse at base line (A) and at end-point (after 30-day of continuous isoproterenol infusion) (B), showing increased excursion during spontaneous breathing. Figures in the excursion scale in the right side of (A) and (B) are mm. (C) Δ indicates the change in diaphragm excursion from baseline to day 30 after starting isoproterenol infusion in HF and in healthy control animals. Values are mean ± SEM. **: $p<0.01$.

https://doi.org/10.1371/journal.pone.0236923.g001

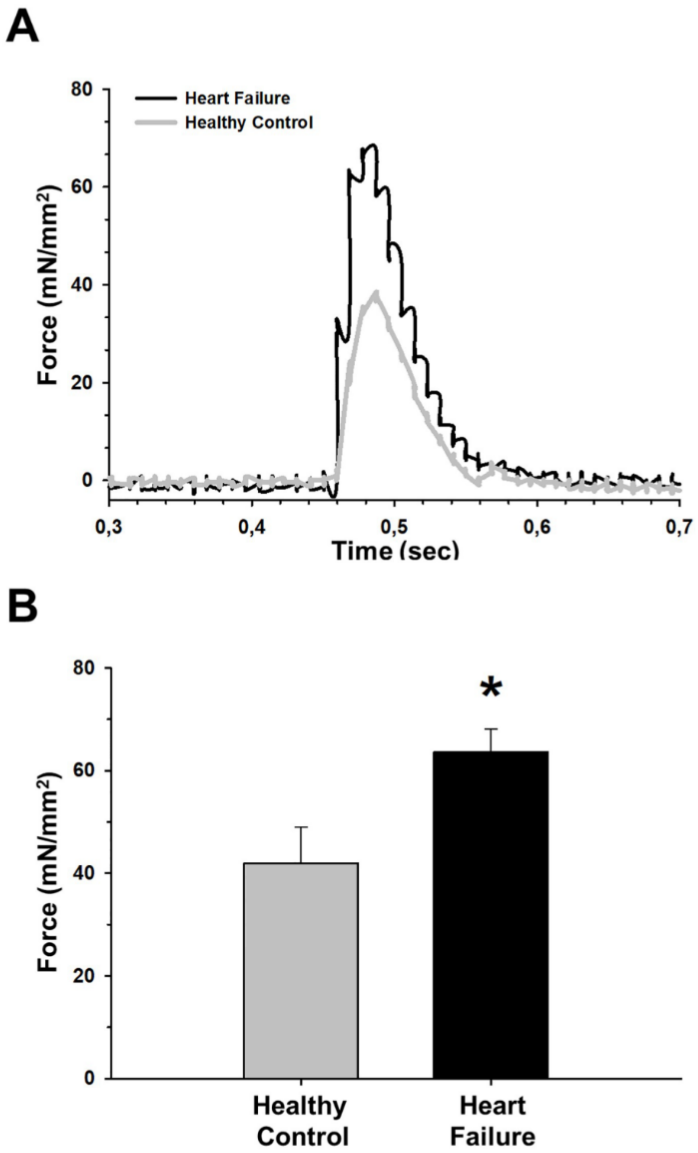

Fig 2. Diaphragm contractile force in a single supramaximal twitch is improved in the isoproterenol-induced heart failure (HF) model. (A) Representative examples of force recordings from healthy (gray) and HF (black) groups. (B) HF animals showed an increase in peak force with respect to healthy mice. Values are mean ± SEM. *: $p<0.05$.

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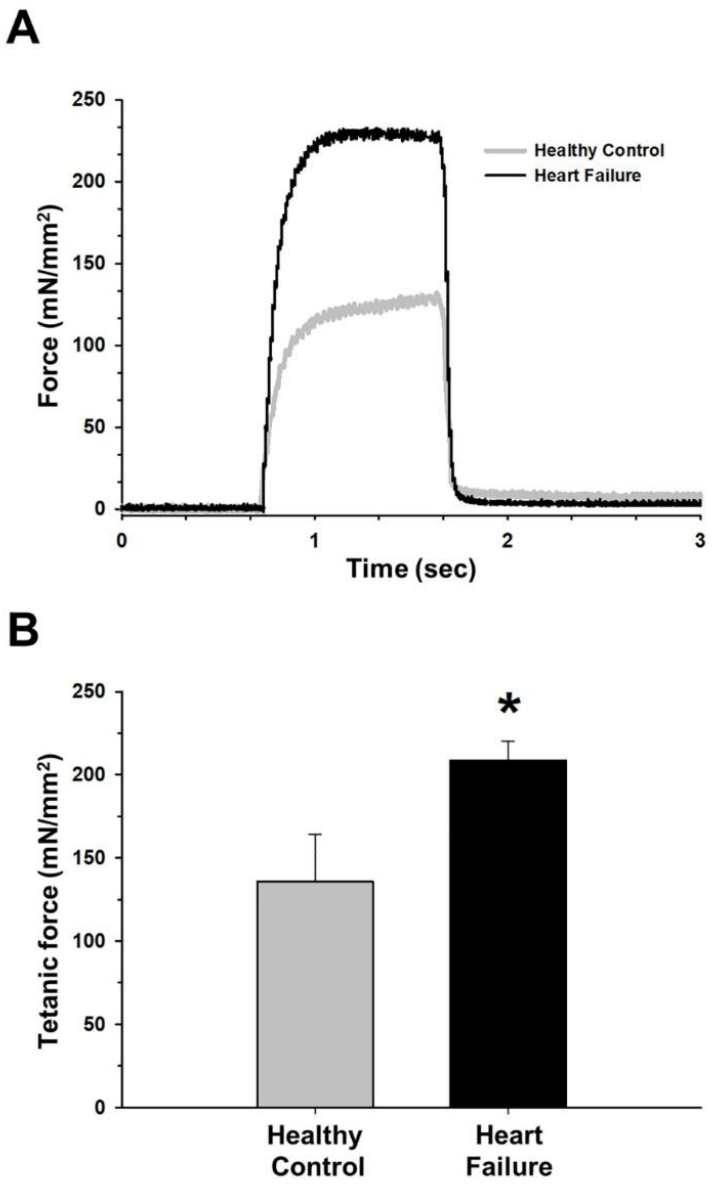

Fig 3. Diaphragm contractile force in a continuous stimulus to generate tetanic contraction is improved in the isoproterenol-induced heart failure (HF) model. (A) Representative examples of tetanic contraction records from healthy (gray) and HF (black) groups. (B) HF animals showed an increase in tetanic force respect healthy group. Values are mean ± SEM. *: $p<0.05$.

https://doi.org/10.1371/journal.pone.0236923.g003

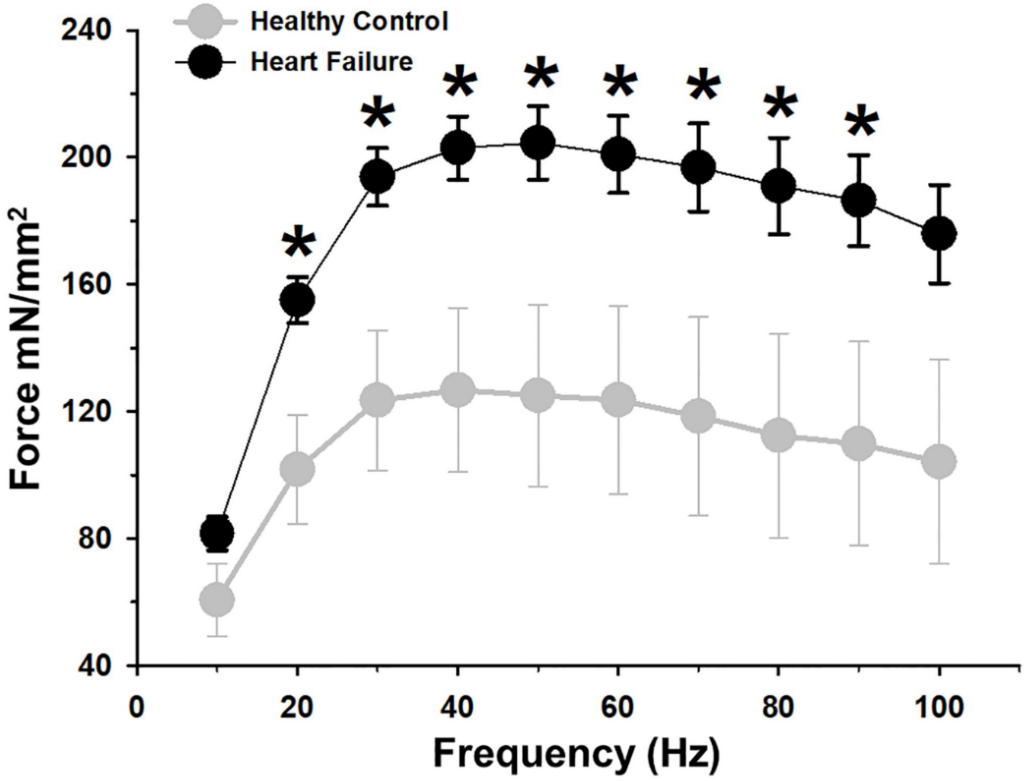

Fig 4. Force frequency relationship between groups at different incremental frequencies. Isoproterenol-induced heart failure (HF) model showed a significant increase in force production for almost all frequencies respect healthy animals. Values are mean ± SEM. *: $p<0.05$.

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duration of isoproterenol application). Accordingly, mice experienced the expected HF structural and functional cardiac changes: increase in LVEDD (by 17%) and in LVESD (by 24%) for structural parameters and decay in functional parameters LVEF (by 12%) and FS (by 18%) as compared with healthy controls. Moreover, HF consistently presented significant lung edema (increase by 29% in the W/D index). Regarding assessment of diaphragm contractibility, we used echography and *ex vivo* specific force measurements. Echography has been validated for detecting time-dependent changes in diaphragmatic function, showing excellent correlation with *ex vivo* force measurement over a wide range of diaphragm excursion values ranging from wild type mice to mutants of Duchenne muscular dystrophy with/out treatment [40]. *Ex vivo* force measurements were carried on diaphragm strips following conventional procedures, achieving values of peak specific twitch and tetanic forces in control mice (≈40 and ≈135 mN/mm^2, respectively) which were very close to the ones reported for wild type mice when using a similar methodology (≈35 and ≈170 mN/mm2, respectively) [41]. This *ex vivo* experimental model allowed us to focus on muscle tissue contractibility thus avoiding the potential effect of isoproterenol on neural activation.

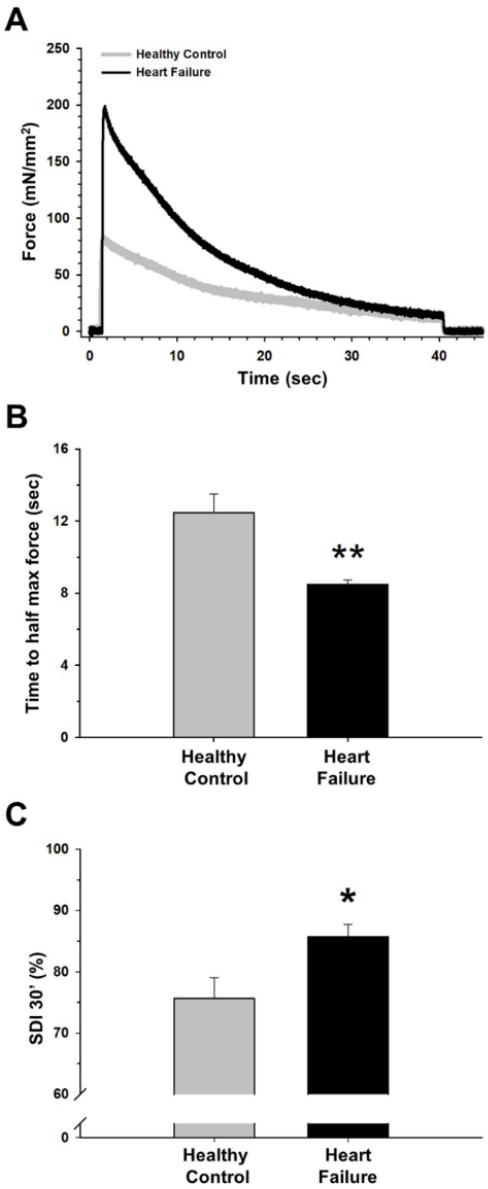

Fig 5. Diaphragmatic fatigue in the isoproterenol-induced Heart Failure (HF) model. (A) Representative examples of fatigue stimulus and decay of force production of healthy and HF diaphragms. (B) HF animals showed a decrease in time to half maximum force and a significant increase in strength decrement index (C) as compared with healthy mice. Values are the mean ± SEM. *: $p<0.05$ and **: $p<0.01$.

https://doi.org/10.1371/journal.pone.0236923.g005

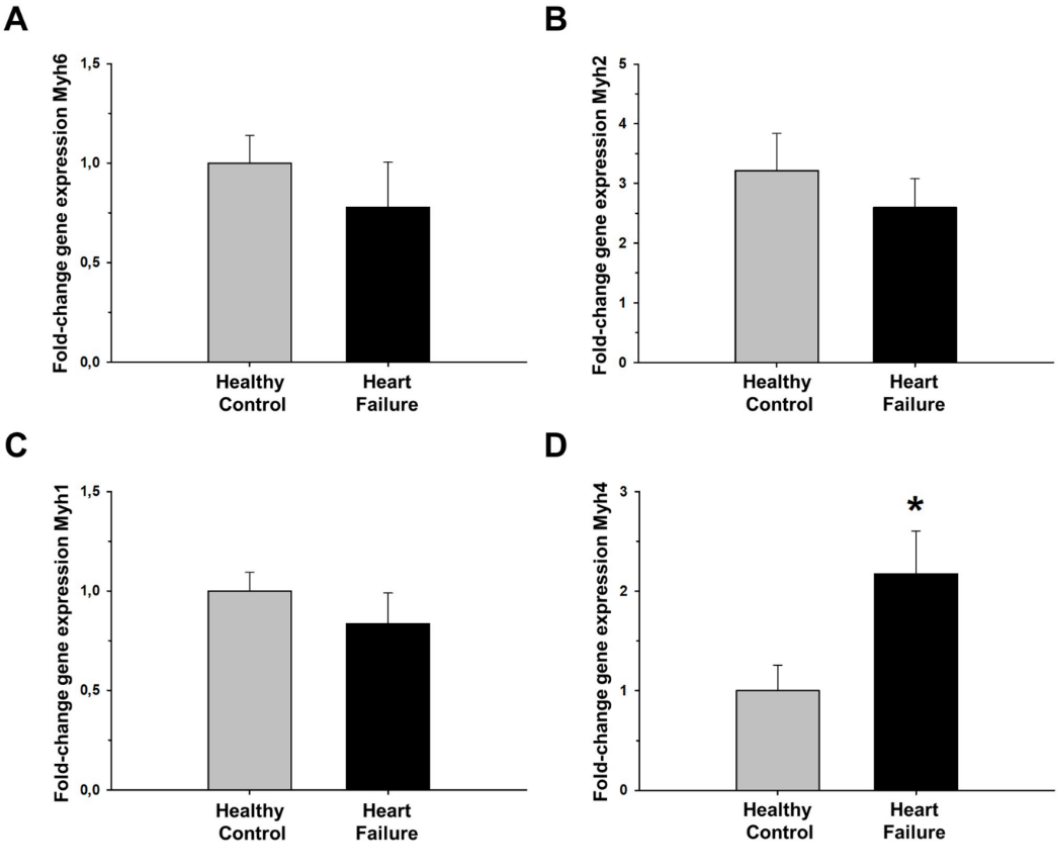

Fig 6. Gene expression of Myh6, Myh2, Myh1 and Myh4 in diaphragm muscle of isoproterenol-induced Heart Failure (HF) mice. (A)Myh6, (B) Myh2, (C) Myh1 and (D) Myh4 as fold-change compared to control healthy mice. Values are the mean ± SEM. *: $p<0.05$.

https://doi.org/10.1371/journal.pone.0236923.g006

The increase in diaphragm contractility in the isoproterenol-induced HF in mice, as compared with healthy controls, was considerable either when assessed noninvasively as diaphragm excursion (by ≈30%) or when measured *ex vivo* (by ≈50% in both peak specific twitch and tetanic forces). Such enhancement in diaphragm function in the isoproterenol model strongly contrasts with findings in other rodent models of HF at similar timepoints. For instance, diaphragm dysfunction has been reported in rat models where HF is induced by left coronary artery ligation [7, 8, 42–45], aorto-caval fistula or aortic banding [46–48] or monocrotaline administration [49]. Regarding mouse models, diaphragm weakening has also been reported when HF is induced by left coronary artery ligation [6, 50–52], transverse aortic constriction [16, 53] or in transgenic mice [54]. Therefore, the novel data reported in the present study on the increased diaphragm contractibility observed in isoproterenol-induced HF at 30 days indicates that, as far as this respiratory muscle function is concerned, the model does not behave as in HF patients and in other rodent models of the disease.

Isoproterenol is a nonselective beta-adrenergic agonist inducing early cardiac hypertrophy and hypercontractility followed by HF with cardiac dilation and ventricular dysfunction secondary to by chronic adrenergic overstimulation [9]. The diaphragm contractility enhancement we report here for the first time in the conventional isoproterenol-induced HF model was hypothesized based on previous indirect data on the effects of this nonselective beta-adrenergic agonist in non-cardiac muscles. Indeed, it was known that the consequences of systemic or global administration of isoproterenol can affect skeletal muscles by activating their beta receptors responsible of fiber contraction. In contrast to the morphological and functional changes described in the heart, chronic stimulation of beta receptors have been shown to prevent muscle atrophy in peripheral skeletal muscle in denervated rats [55]. Also, dietary administration of beta adrenoceptor agonist in rats produce growth-promoting protein anabolic effects in muscle tissue [56]. Chronic administration of these agonists causes hypertrophy of skeletal muscle in mice [57] and hypertrophy of diaphragm in hamsters [58]. Moreover, when investigated in different experimental settings other than the HF model, isoproterenol increased canine and rodent diaphragm contractibility [12–14]. It is interesting to note that isoproterenol may also increase the contractile force of skeletal muscles, as it has been reported when added to the bath of *ex vivo* preparations of mouse soleus and extensor digitorum longus [10, 11]. However, whether continuous chronic infusion of isoproterenol as in the HF mouse model induces enhancement of skeletal muscle contractibility is unknown.

Noteworthy, in addition to production of enhanced force, the diaphragm of isoproterenol-induced HF failure mice also showed fast decrease rate and hence a tendency to fatigue (Fig 5). Interestingly, an increase in force production and less fatigue resistance are both characteristic features of muscles with a greater distribution of type IIb fibers [59–61]. The significant increase in *Myh4* (related to MyHC-IIb, the most fast-glycolytic muscle fiber type) in our HF mice (Fig 6) could partially explain the results observed in the *ex vivo* muscle testing. This fiber distribution contrasts with the results reported from diaphragm biopsies in patients with severe HF, showing a shift from fast to slow fibers with higher levels of type I and lower levels of type II fibers compared with healthy controls [62]. Likewise, in animal models of chronic HF that adequately reproduce diaphragmatic weakness some studies also described the same tendency with increase in type I and IIa muscle fibers accompanied by decreases in type IId/x and IIb fibers [43, 47, 63]. The shift from a more fast-glycolytic to a slow-oxidative metabolism in HF partially explains diaphragm weakness and his relationship with breathlessness/dyspnea and exercise intolerance. Therefore, although more detailed fiber analysis could be carried out, our results from myosin gene expression in diaphragms from isoproterenol-induced HF (Fig 6) suggest that changes in fiber types differ from those described in HF patients and explain the greater force production together with the greater vulnerability to fatigue.

This study has some limitations. First, to selectively identify the effect of isoproterenol, a subcutaneous pump releasing saline was implanted into the mice in the healthy control group. It should be noted, however, that implantation of such a subcutaneous pump is a minor procedure with very unlikely systemic consequences. Second, as we mainly focused on documenting the existence of isoproterenol-induced diaphragm increase in contractile force, we did not focus on involved mechanisms, such as whether force production correlated to Ca+2 handling, or on ultrastructural analysis of the diaphragm fibers. the HF model was evaluated at 30 days of continuous isoproterenol infusion, which is a previously validated method [64]. Whether further beta-adrenergic stimulation during longer periods of time could lead to greater vulnerability to diaphragm fatigue and secondary dysfunctional contractility (reproducing the effects of chronic beta-stimulation at the heart) is unknown. However, our preliminary data showing mRNA expression of myosin genes diverging from those described in other HF models with similar timepoints and in HF patients do not support this hypothesis. Interestingly, detailed

analysis at different time points in the model would allow characterizing the progression of the diaphragmatic alterations and the correlation between the magnitude of diaphragm contractibility and the severity of heart failure [14].

Conclusions

In summary, this study has demonstrated a previously unreported and relevant limitation of the conventional rodent model of isoproterenol-induced HF. Indeed, whereas this model is suitable for mimicking the cardiac structural and functional alterations in HF, its considerable increase in diaphragm contractibility is the reverse of the diaphragmatic weakening observed in patients with this disease, thereby questioning its translational interest in HF research, especially when aimed at integrating cardiorespiratory alterations.

Acknowledgments

The authors wish to thank Mr. Miguel Rodriguez and Ms. Elisabet Urrea for their excellent technical assistance.

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Funding acquisition: Ramon Farré.

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Chapter V:
SCIENTIFIC ARTICLE II

Impact of and Reasons for Not Performing Exercise Training After an Acute Coronary Syndrome in the Setting of an Interdisciplinary Cardiac Rehabilitation Program: Results From a Risk-Op-Acute Coronary Syndrome Ambispective Registry

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Front. Physiol., vol. 12, pp. 1–8, Nov. 2021.

IF = 4.755, Q1/T1 (20/81)

frontiers in Physiology

ORIGINAL RESEARCH
published: 24 November 2021
doi: 10.3389/fphys.2021.768199

Impact of and Reasons for Not Performing Exercise Training After an Acute Coronary Syndrome in the Setting of an Interdisciplinary Cardiac Rehabilitation Program: Results From a Risk-Op- Acute Coronary Syndrome Ambispective Registry

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Background and Aims: Exercise training (ET) is a critical component of cardiac rehabilitation (CR), but it remains underused. The aim of this study was to compare clinical outcomes between patients who completed ET (A-T), those who accepted ET but did not complete it (A-NT), and those who did not accept to undergo it (R-NT), and to analyze reasons for rejecting or not completing ET.

Methods and Results: A unicenter ambispective observational registry study of 497 patients with acute coronary syndrome (ACS) was carried out in Barcelona, Spain, from 2016 to 2019. The primary endpoint was a composite of all-cause mortality, hospitalization for ACS, or need for revascularization during follow-up. Multivariable analysis was carried out to identify variables independently associated with the primary outcome. Initially, 70% of patients accepted participating in the ET, but only 50.5% completed it. The A-T group were younger and had fewer comorbidities. Baseline characteristics in A-NT and R-NT groups were very similar. The main reason for not undergoing or completing ET was rejection (reason unknown) or work/schedule incompatibility. The median follow-up period was 31 months. Both the composite primary endpoint and mortality were significantly lower in the A-T group compared to the A-NT and R-NT (primary endpoint: 3.6% vs. 23.2% vs. 20.4%, $p < 0.001$, respectively;

OPEN ACCESS

Edited by:
Maria Fernandez-del-Valle, Universidad Isabel I, Spain

Reviewed by:
Tamara Iturriaga, European University of Madrid, Spain
Garry Tew, Northumbria University, United Kingdom

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *31 August 2021*
Accepted: *31 October 2021*
Published: *24 November 2021*

Citation:
Cabrera-Aguilera I, Ivern C, Badosa N, Marco E, Salas-Medina L, Mojón D, Vicente M, Llagostera M, Farré N and Ruiz-Bustillo S (2021) Impact of and Reasons for Not Performing Exercise Training After an Acute Coronary Syndrome in the Setting of an Interdisciplinary Cardiac Rehabilitation Program: Results From a Risk-Op- Acute Coronary Syndrome Ambispective Registry. Front. Physiol. 12:768199. doi: 10.3389/fphys.2021.768199

mortality: 0.8% vs. 9.1% vs. 8.2%, $p < 0.001$; respectively). During multivariable analysis, the only variables that remained statistically significant with the composite endpoint were ET completion, previous ACS, and anemia.

Conclusion: Completion of ET after ACS was associated with improved prognosis. Only half of the patients completed the ET program, with the leading reasons for not completing it being refusal (reason unknown) and work/schedule incompatibility. These results highlight the need to focus on the needs of patients in order to guarantee that structural barriers to ET no longer exist.

Keywords: acute coronary syndrome, cardiac rehabilitation, exercise training, rehabilitation adherence, ischemic heart disease, event-free survival

INTRODUCTION

According to the World Health Organization, ischemic heart disease was the top cause of death in 2000 and 2019, responsible for 16% of total deaths each year. Moreover, fatalities due to the disease have seen the most significant increase, rising from over 2 million in 2000 to nearly 9 million in 2019 (Nowbar et al., 2019; World Health Organization [WHO], 2021). Despite the improvements in primary prevention, diagnosis, and treatments (Widimsky et al., 2019), the long-term prognosis of ischemic heart disease remains poor, with a high rate of acute coronary syndrome (ACS), need for coronary revascularization, and death (Collet et al., 2021). Cardiac rehabilitation (CR) is an integral and complex intervention that comprises exercise training (ET), behavioral change, psychological support, and other strategies to control traditional risk factors of cardiovascular disease. Several studies and meta-analyses have shown that CR is associated with better prognosis. Therefore, CR has a class I recommendation from the European Society of Cardiology for the management of a cardiac ischemic event (Anderson et al., 2016; Ibanez et al., 2018; Ji et al., 2019; Collet et al., 2021). Unfortunately, CR remains underused due to multiple reasons (Bethell et al., 2008; Balady et al., 2011; Serón et al., 2019). On the one hand, many countries and regions do not have CR programs. On the other hand, patients are often unwilling or unable to enroll in CR, especially the ET component. Indeed, only 50% of patients referred for CR end up participating in ET (Dunlay et al., 2014). Although several studies have analyzed the difference between patients who accept to undergo ET and those who do not, few studies have studied patients who begin ET but do not complete it. Thus, it is unknown whether patients who begin ET but do not complete it have different baseline characteristics and outcomes than patients who complete ET and those who outrightly reject it. Patient-centered care (PCC) has been proposed as a central component for a sustainable, affordable, and high-quality healthcare approach. It underlines the importance of understanding the patient's capabilities and resources in order to engage the patient to participate in care (Ekman et al., 2011). PCC efficiency has been demonstrated in several conditions and care levels, including ACS (Fors et al., 2016; Wolf et al., 2019). To provide optimal PCC, it is critical to know why patients choose not to participate in CR. This knowledge would improve prognosis.

This study aimed to assess whether there were epidemiological differences between patients referred for CR according to exercise compliance and initial attitude toward ET, analyze the reasons for rejecting or not completing ET, and explore the prognostic impact of each group.

MATERIALS AND METHODS

Study Design and Population

A total of 497 patients from an ambispective observational registry study carried out at the Hospital del Mar, Barcelona, Spain from November 2016 to September 2019 were included in this analysis. Patients with ST-elevation acute myocardial infarction (STEMI), non-ST-elevation acute myocardial infarction (non-STEMI), and unstable angina (UA) were included. The diagnosis was made according to the European Heart Association guidelines (Roffi et al., 2015; Ibanez et al., 2018). All patients from our health area were invited to participate in the CR program (CRP). The only exclusion criteria were being from other health areas and the existence of a severe language barrier.

Cardiac Rehabilitation Program

The CRP at the Hospital del Mar is an interdisciplinary program that combines interventions performed by cardiologists, nurses, rehabilitation physicians, physiotherapists, and psychiatrists. All patients with ACS receive education on healthy habits during the initial ACS hospitalization, or shortly after discharge for those hospitalized in other hospitals. Specialized nurses undertake follow-up visits 3 and 12 months after inclusion in the CRP. Patients attend weekly group sessions with healthcare professionals aimed at reinforcing their health education, with a particular focus on understanding the pathophysiology of ACS, the role of cardiovascular risk factors, and the importance of optimal risk factor management, mainly through physical activity, control of anxiety, smoking cessation, and adherence to guideline-recommended drugs.

All patients are referred to participate in ET. Patient functional status is assessed on enrollment by a treadmill test. Rehabilitation physicians prescribe the levels and types of exercise for patients according to their characteristics and risk stratification. The ET

intervention consists of 25 1-h sessions—five times per week (Monday–Friday from 9 to 10 a.m.) for 5 weeks. Each session starts with a 5-min warm-up period followed by 30 min of cycling (80% of maximal heart rate achieved in an exercise test), 20 min of resistance muscle training of upper and lower limbs (weight at 10 RM), and a 5-min cool-down period. An expert physiotherapist supervises sessions with continuous heart rate and pulse oximetry monitoring. The workload progression is adjusted weekly according to the patient's tolerance (Borg perceived effort scale). Dropout in ET is defined as attending no more than 50% of sessions (Worcester et al., 2004).

Study Variables

We collected baseline demographic and clinical data and follow-up events, viz.: age, gender, BMI, risk factors and comorbidities, diagnostics, and basal hospitalization data. Patients were classified into three groups: those who completed the ET (A-T), those who outrightly rejected ET (R-NT), and those who accepted but did not complete ET (A-NT). We collected the reasons for not wanting to perform the ET. When the cause was not established in the medical record, it was classified as "rejected-reason unknown." The primary endpoint was a frequently used composite of all-cause mortality, hospitalization due to ACS, or need for new revascularization during follow-up (Bainey et al., 2020; Turgeon et al., 2020).

Statistics

Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) based on normality distribution assessed by the Kolmogorov–Smirnov test. Categorical variables were expressed as percentages. Differences in baseline characteristics between groups previously defined by level participation in CRP were tested using the χ^2-test (categorical variables); and Student's *t*-test or the Mann–Whitney *U*-test, one-way analysis of variance or the Kruskal–Wallis test for continuous variables. Univariable and multivariable analyses were performed using the Cox proportional hazard regression model to examine the association between ET participation and outcomes. Variables with an overall significance value of $p < 0.05$ were used for multiple Cox regression analysis to identify the strongest predictors of event-free survival. The model was adjusted for potential confounders selected by stepwise forward inclusion among patient characteristics previously defined. The variables included in the multivariable analysis have also been associated with the endpoint in several studies (Arnold et al., 2015; Bainey et al., 2020; Turgeon et al., 2020). The log-rank test compared the Kaplan–Meier survival curves. All analysis was performed using IBM SPSS Statistics v25 (Armonk, NY, United States) and GraphPad Prism 8.0 (San Diego, CA, United States). For all tests, $p < 0.05$ was considered as statically significant.

Ethics

The study was designed in compliance with the ethical principles set forth in the Declaration of Helsinki. The Ethics Committee of the Hospital del Mar (Parc de Salut Mar) approved the study (N° 2018/7896/I). The data included in this study incorporated data from a registry collection carried out prospectively between July 2018 and September 2019, with all the patients providing written informed consent. Retrospective data collection was carried out between November 2016 and June 2018, with the Ethical Committee approving the inclusion of these group of patients to increase the sample size but waiving the need for written informed consent by this group, given that the same protocol had been carried out before.

RESULTS

Patients' Baseline Clinical Characteristics

The flow chart of the 497 patients included in this study is depicted in **Figure 1**. Although 70% of patients initially accepted to participate in the ET, 28% of this group did not complete the ET for various reasons. Therefore, only 50.5% of the whole population completed the ET.

Baseline clinical characteristics of the study population are summarized in **Table 1**. In brief, most of the patients were men with a diagnosis of STEMI, 1-vessel disease, and preserved ejection fraction. No significant differences were found when comparing both sexes. Patients who completed ET (A-T) were younger and had fewer comorbidities. Patients in R-NT and A-NT groups had similar baseline characteristics.

Reasons for Not Completing the Exercise Training Component of the CR Program

The main reason for outrightly rejecting to participate in or not completing the ET was rejection-reason unknown. **Table 2** presents the reasons for non-participation in and non-completion of the ET component of the CRP. Reasons for not completing the ET were qualitatively somewhat different in women and men.

Only four patients (4% of A-NT) could not complete the ET due to cardiovascular complications: one patient with atrial fibrillation, one with a high-risk treadmill test, one due to chest pain, and one with ACS. These events did not occur during the ET. Work incompatibility and distance/schedule were also relevant reasons for not performing ET in both the A-NT and R-NT groups. It is noteworthy that none of the A-T patients experienced a CV complication while exercising, confirming that ET was safe in this group of patients.

Prognosis

During a median follow-up of 31 (23–39) months, 12% of all patients showed a composite outcome of all-cause death, new ACS, or need for coronary revascularization. The A-T group showed an excellent prognosis. In this group, the mortality rate was < 1%. The prognoses in the A-NT and R-NT groups were similar and worse than that in the A-T group (**Table 3**).

After adjustment for age, sex, anemia, hypertension, diabetes mellitus, renal disease, previous ACS, peripheral vascular disease, type of ACS, and ET compliance, the only variables that remained statistically significant with the composite endpoint on

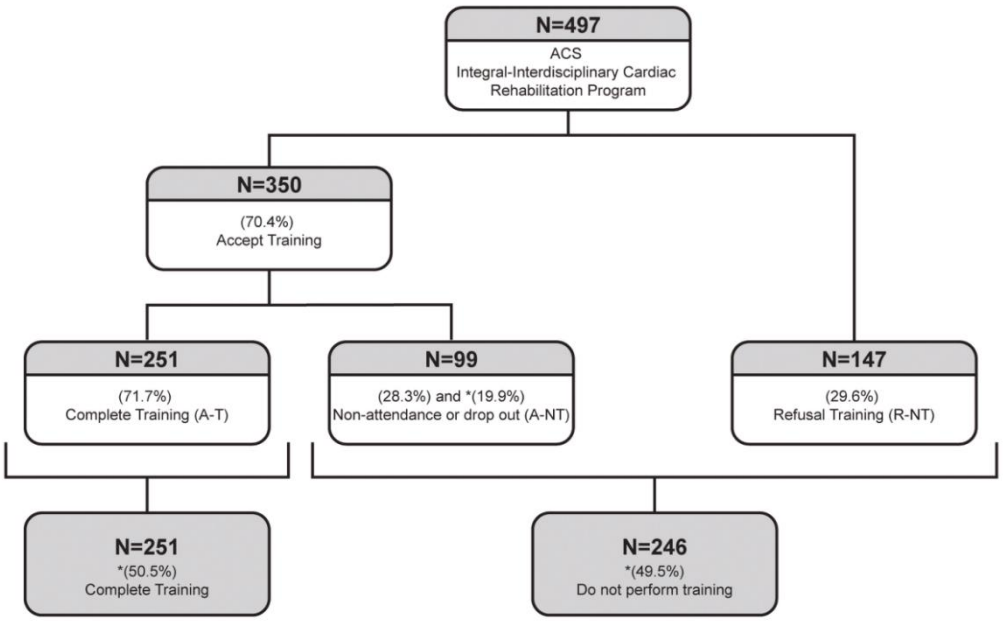

FIGURE 1 | Flow chart of patients in the cardiac rehabilitation program. ACS, Acute coronary syndrome; R-NT, Rejected and did not perform training; A-T, Accepted and did exercise training; A-NT Accepted but did not do/complete training; CV, Cardiovascular. Data are expressed in percentages to the preceding category. Data with an asterisk (*) were calculated to refer to the whole cohort.

multivariable Cox regression analysis were ET compliance [A-NT, HR 5.6 (95% CI 2.75–13.05); R-NT, HR 4.32 (2.00–9.29)], previous ACS and anemia (**Table 4**).

Figure 2 shows the Kaplan–Meier survival curves for the composite endpoint and all-cause mortality in the three groups; the A-T group shows better survival in both curves.

DISCUSSION

Although 70% of patients accepted to participate in ET after ACS during a CRP, only 50.5% of the total patients completed the ET protocol. Patients who completed ET (A-T) were younger and had fewer comorbidities. Their prognosis was excellent, with mortality < 1% at a median follow-up of 31 months. Patients who did not complete ET (A-NT and R-NT) had similar baseline characteristics and prognosis, which was worse than that of the A-T group. The main reason for not participating in ET was refusal from the patients and work and schedule/distance incompatibilities, highlighting the need to find ways to engage patients in ET and increase ET logistic capabilities.

Although the initial number of patients who accepted ET was 70%, only 50.5% completed the ET protocol, a rate similar to the 52.5% participation obtained in a population-based surveillance study of patients after their first ever myocardial infarction (Dunlay et al., 2014). Patients who completed the ET were younger and with fewer comorbidities, observations that are consistent with those of other studies (Dunlay et al., 2014; Pardaens et al., 2017). This finding might seem counterintuitive because higher-risk patients (i.e., those with comorbidities) should be more prone to accept ET to decrease their risk (Bainey et al., 2020). Several reasons might explain why this was not the case in this study. On the one hand, patients who completed ET (A-T) might be fitter due to their being younger and having fewer comorbidities, and more open to going to the gym to exercise. Moreover, this group had less history of ACS and presented more frequently with STEMI. This acute presentation might have prompted them to accept any option that would improve their prognosis. On the other hand, the A-NT and R-NT patients were older and had more comorbidities, some of which might have hindered their ability to exercise (e.g., peripheral vascular disease and anemia), and even though the exercise was adapted to the needs of the patients, some patients might not have found it valuable and thus rejected it. Surprisingly, there is not much information in the literature on patients who initially accept to undergo ET but do not start or finish it. Given that they accounted for 28% of the patients who initially accepted to undergo ET in this study, it is a group that should not be overlooked. Their baseline characteristics were similar to those of the R-NT group except for a lower prevalence of peripheral vascular disease.

Reasons for low rates of use of CR are very diverse among different countries and regions. Barriers to participation in ET are classified as inherent to the patient's characteristics or related to accessibility of the CRP system (Ruano-Ravina et al., 2016). For instance, gender, age, comorbidities, disease perception, social class, education level or accessibility, and proximity to

TABLE 1 | Baseline characteristics of patients in the cardiac rehabilitation program and in the three groups.

Characteristic/Variable	Overall (*n* = 497)	A-T (*n* = 251)	A-NT (*n* = 99)	R-NT (*n* = 147)	*p*-value*	*p*-value**	*p*-value***	*p*-value****
Anthropometric								
Age (years)	62.6 ± 11.9	60.9 ± 10.7	62.5 ± 11.2	65.6 ± 13.6	0.001	0.203	0.056	<0.001
Women	93 (18.7)	45 (17.9)	16 (16.2)	32 (21.8)	0.490	0.695	0.276	0.349
BMI (Kg/m^2)	27.1 (24.7–30.1)	27.0 (24.7–29.4)	27.3 (23.9–30.9)	27.0 (24.6–30.4)	0.781	0.523	0.901	0.618
Risk factors and comorbidities								
Hypertension	281 (56.5)	126 (50.2)	58 (58.6)	97 (66.0)	0.008	0.157	0.238	0.002
Hyperlipidemia	324 (65.2)	168 (66.9)	59 (59.6)	97 (66.0)	0.419	0.195	0.308	0.847
Diabetes mellitus	143 (28.8)	55 (21.9)	32 (32.3)	56 (38.1)	0.002	0.042	0.354	0.001
Current smoker	205 (41.3)	95 (37.9)	46 (46.5)	64 (43.5)				
Previous smoker > 1 year	146 (29.4)	82 (32.7)	25 (25.3)	39 (26.5)	0.705	0.406	0.953	0.585
Previous smoker < 1 year	20 (4.0)	11 (4.4)	3 (3.0)	6 (4.1)				
COPD	40 (8.1)	15 (6.0)	10 (10.0)	15 (10.2)	0.230	0.177	0.979	0.123
Cerebrovascular disease	23 (4.6)	9 (3.6)	5 (5.1)	9 (6.1)	0.496	0.529	0.722	0.240
Peripheral vascular disease	39 (7.9)	14 (5.6)	3 (3.0)	22 (15.0)	<0.001	0.318	0.002	0.002
Anemia	87 (17.5)	29 (11.6)	20 (20.2)	38 (25.9)	0.001	0.036	0.306	<0.001
Renal disease	29 (5.8)	8 (3.2)	5 (5.1)	16 (10.9)	0.006	0.406	0.108	0.002
Arthropathy	10 (2.0)	2 (0.8)	2 (2.0)	6 (4.1)	0.079	0.332	0.371	0.024
Diagnostics and basal hospitalization								
STEMI	220 (44.3)	120 (47.8)	41 (41.4)	59 (40.1)				
NSTEMI	174 (35.0)	93 (37.1)	38 (38.4)	43 (29.3)	0.007	0.414	0.139	0.001
Unstable angina	103 (20.7)	38 (15.1)	20 (20.2)	45 (30.6)				
Hospitalization length (days)	5 (4–7)	5 (3–6)	5 (4–7)	5 (4–7)	0.116	0.169	0.816	0.055
Previous ACS-MI	99 (19.9)	34 (13.6)	21 (21.2)	44 (29.9)	<0.001	0.076	0.128	<0.001
One vessel disease	263 (52.9)	144 (57.4)	42 (42.4)	77 (52.4)				
Two vessel disease	126 (25.4)	59 (23.5)	34 (34.3)	33 (22.5)	0.192	0.138	0.200	0.474
Three vessel disease	87 (17.5)	40 (15.9)	20 (20.2)	27 (18.4)				
Ejection fraction (%)	58 (52–62)	58 (51–62)	60 (52–63)	59 (54–62)	0.451	0.545	0.695	0.210

Data are mean ± SD, median (IQR), or n (%). p values for comparing all three groups (null hypothesis: all three groups had the same characteristics). p** values only apply to the comparison of Accepted and did exercise training (A-T) vs. Accepted but did not do/complete training (A-NT) groups. p*** values only apply to the comparison of A-NT vs. Rejected and did not perform training (R-NT) groups. p**** values only apply to the comparison of A-T vs. R-NT groups.*
BMI, Body mass index; COPD, chronic obstructive pulmonary disease; CV, Cardiovascular; STEMI, ST-elevation myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; ACS-MI, Acute coronary syndrome-myocardial infarction.

TABLE 2 | Description of reasons by sexes for not doing the exercise training in the group that initially refused and those who initially accepted but did not complete exercise training.

R-NT (*n* = 147)	Female (*n* = 32)	Male (*n* = 115)	All	A-NT (*n* = 99)	Female (*n* = 16)	Male (*n* = 83)	All
Rejection-reason unknown	12 (37.5)	45 (39.1)	57 (38.8)	Rejection-reason unknown	6 (37.5)	38 (45.8)	44 (44.4)
Functional problems	6 (18.8)	16 (13.9)	22 (15.0)	Medical recommendation	8 (50.0)	21 (25.3)	29 (29.3)
Job/work incompatibility	7 (21.9)	14 (12.2)	21 (14.3)	Job/work incompatibility	2 (12.5)	17 (20.5)	19 (19.2)
Schedule and distance	1 (3.1)	20 (17.8)	21 (14.3)	CV disease	0 (0.0)	4 (4.8)	4 (4.0)
Medical recommendation	4 (12.5)	8 (7.0)	12 (8.2)	Schedule and distance	0 (0.0)	2 (2.4)	2 (2.0)
Language barrier	0 (0.0)	9 (7.8)	9 (6.1)	SARS-CoV-2 contingency	0 (0.0)	1 (1.2)	1 (1.0)
Social problems	2 (6.3)	3 (2.6)	5 (3.4)				

Data are n (%). CV, Cardiovascular.

the CRP center plays a crucial role in adherence to ET. For women, barriers to CR participation are multiple and complex, explaining their low participation rates (Forsyth and Deaton, 2020; Vidal-Almela et al., 2020). While our study included 18.71% of female, some studies reported an 11–20% lower enrollment, and women were more likely to withdraw from a program than men (35% vs. 29%) (Marzolini et al., 2008; Balady et al., 2011). Reasons for not accepting to undergo ET, or for accepting but not completing it, were quite similar in the R-NT and A-NT groups. The main reason in both groups (41% of patients) was the unwillingness of the patients to participate, with no explanations given for this unwillingness. This finding is consistent with those of other studies where 47.5% of patients did not participate in ET (Worcester et al., 2004; Dunlay et al., 2014). The other reasons

TABLE 3 | Outcomes of patients included in the cardiac rehabilitation program and in the three groups.

Outcome	Overall (*n* = 497)	A-T (*n* = 251)	A-NT (*n* = 99)	R-NT (*n* = 147)	*p*-value*	*p*-value**	*p*-value***	*p*-value****
Composite Outcome	62 (12.5)	9 (3.6)	23 (23.2)	30 (20.4)	< 0.001	< 0.001	0.597	< 0.001
Death	23 (4.6)	2 (0.8)	9 (9.1)	12 (8.2)	< 0.001	< 0.001	0.798	< 0.001
New ACS	25 (5.0)	5 (2.0)	7 (7.1)	13 (8.8)	0.006	0.019	0.618	0.002
Revascularization	36 (7.2)	8 (3.2)	12 (12.1)	16 (10.9)	0.002	0.001	0.765	0.002
All-cause hospitalization	168 (33.8)	61 (24.3)	48 (48.5)	59 (40.1)	< 0.001	< 0.001	0.195	0.001
All-CV hospitalization	81 (16.3)	22 (8.8)	19 (19.2)	40 (27.2)	< 0.001	0.006	0.149	< 0.001

ACS, Acute coronary syndrome; CV, Cardiovascular. Data are n (%). p values for comparing all three groups (null hypothesis: all three groups had the same characteristics). p** values only apply to the comparison of Accepted and did exercise training (A-T) vs. Accepted but did not do/complete training (A-NT) groups. p*** values only apply to the comparison of A-NT vs. Rejected and did not perform training (R-NT) groups. p**** values only apply to the comparison of A-T vs. R-NT groups.*

TABLE 4 | Univariable and multivariable Cox regression analyses for the composite endpoint.

	Univariable HR (95% CI)	*p*-value	Adjusted HR (95% CI)	*p*-value
A-T	1 (ref)		1 (ref)	
A-NT	7.12 (3.30–15.40)	< 0.001	5.60 (2.75–13.05)	< 0.001
R-NT	5.88 (2.79–12.38)	< 0.001	4.32 (2.00–9.29)	< 0.001
Previous ACS-MI	2.79 (1.68–4.63)	< 0.001	1.81 (1.07–3.08)	0.028
Anemia	3.48 (2.09–5.78)	< 0.001	2.33 (1.37–3.97)	0.002
Renal disease	2.96 (1.46–6.01)	0.003		
Age (years)	1.04 (1.02–1.06)	0.001		
Hypertension	1.97 (1.14–3.42)	0.015		
Diabetes mellitus	2.02 (1.23–3.34)	0.006		
Female	1 (ref)			
Male	1.22 (0.66–2.25)	0.521		
Peripheral vascular disease	1.33 (0.57–3.10)	0.504		
STEMI	1 (ref)			
NSTEMI	1.43 (0.79–2.58)	0.241		
Unstable angina	1.88 (1.00–3.53)	0.050		

The composite endpoint includes all-cause mortality, hospitalization due to ACS, or the need for new revascularization during follow-up. A-T, Accepted and did exercise training; A-NT, Accepted but did not do/complete training; R-NT, Rejected and did not perform training (R-NT) groups; STEMI, ST-elevation myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; ACS-MI, Acute coronary syndrome-myocardial infarction.

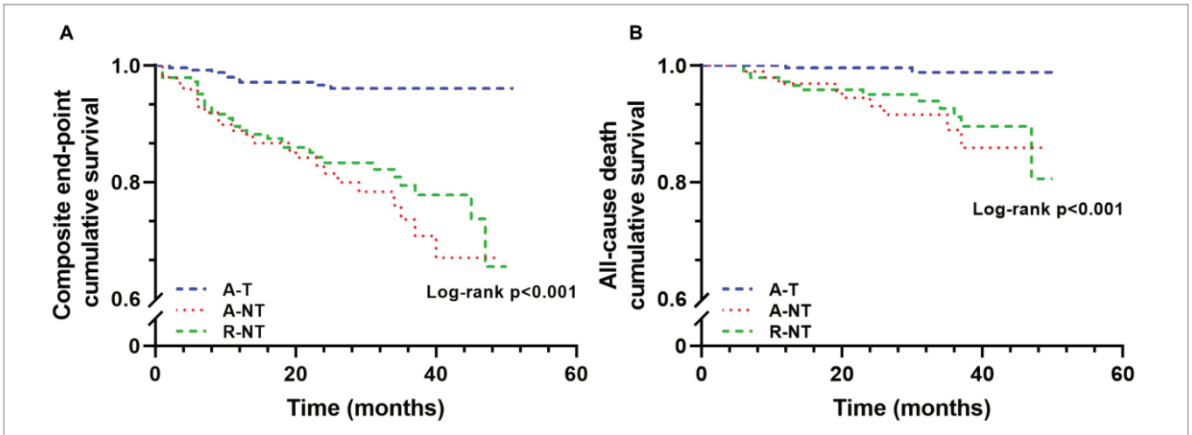

FIGURE 2 | Kaplan–Meier curves for long-term outcome grouped by ET groups (**A**, composite endpoint cumulative survival; **B**, cumulative survival). A-T, Accepted and did exercise training; A-NT, Accepted but did not do/complete training; R-NT, Rejected and did not perform training. The composite endpoint includes all-cause mortality, hospitalization due to ACS, or the need for new revascularization during follow-up.

more frequently mentioned were job/work incompatibility and schedule and distance, which accounted for 26% of patients that did not participate or withdrew from ET. Reasons for not completing ET were different between men and women. They also varied according to the time of assessment (initial refusal vs. once accepted but not completed), emphasizing the need to tailor ET to the needs of patients. Only 27% of patients were excluded from ET due to medical reasons, such as the presence of comorbidities. This percentage is significant and highlights the need to focus on what can be changed in the system to guarantee that structural barriers to ET disappear, so that genuine PCC can be carried, which would greatly increase the number of patients who complete ET and also bridge the gender gap in participation. PCC can help identify intangible barriers, such as those reported by Resurrección et al. (2017), in which traditional ways of CRP with predominantly male presence could make women feel uncomfortable. Finally, our results showed an excellent long-term prognosis in patients who completed ET, with a composite of all-cause mortality, hospitalization due to ACS, or need for new revascularization in only 3.6%, after more than 2 years of follow-up, and a mortality of 0.8%. In contrast, patients in the R-NT and A-NT groups showed at least four times more risk with the composite endpoint and all-cause mortality, with no significant difference between the two groups. These results are consistent with those of other studies that found that the risk of death or having a recurrent CV event in patients that do not complete ET is over double the risk in patients who complete it (Pardaens et al., 2017).

LIMITATIONS

As a single-center study, the results might not apply to other settings. Some of the patients were included retrospectively, and that might have potentially led to bias. Still, given that all patients followed the same protocol and that the information was documented in the medical record in a structured way, we believe that the risk of bias in this study is negligible. The lack of ET schedule flexibility in our center might have affected adherence. Patients' participation might have been higher if we had more liberty to schedule their ET time. Due to the low number of women in our sample size, we cannot comment on the gender differences in ET participation.

CONCLUSION

Completion of ET after ACS is associated with an improved prognosis. Only half of the patients in this registry study completed the ET component in the setting of an interdisciplinary CRP, although 70% of patients accepted participating in ET. Patients who completed ET (A-T) were younger and had fewer comorbidities. Their prognosis was excellent, with mortality < 1% at a median follow-up of 31 months. Patients who outrightly rejected participating in ET and those who began but did not complete it had similar baseline characteristics and prognosis, which was worse than that of the A-T group. The main reasons for not participating in AT in both groups were refusal-reason unknown and work and schedule/distance incompatibility. These results highlight the need to focus on the needs of the patients in order to minimize structural barriers to ET.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The study was designed in compliance with the ethical principles set forth by the Declaration of Helsinki. The Ethics Committee of the Hospital del Mar (Parc de Salut Mar) approved the study (N° 2018/7896/I). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SR-B and NB contributed to the conception or design of the work. NB, CI, MV, ML, DM, and IC-A contributed to the data acquisition. NF and IC-A performed data analysis and drafted the manuscript. NF, SR-B, and IC-A contributed to data interpretation. EM, SR-B, and LS-M critically revised the manuscript. All authors approved the final version of the manuscript and are accountable for all aspects of the work.

FUNDING

IC-A was supported by the ANID-PFCHA—Chilean Doctorate Fellowship 2017; Grant No. 72180089.

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Chapter VI:
SCIENTIFIC ARTICLE III

Prognostic Utility of a New Risk Stratification Protocol for Secondary Prevention in Patients Attending Cardiac Rehabilitation

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J. Clin. Med., vol. 11, pp. 1910–21, Mar 2022.

IF = 4.964, Q2/T1 (54/172)

Journal of Clinical Medicine

MDPI

Article

Prognostic Utility of a New Risk Stratification Protocol for Secondary Prevention in Patients Attending Cardiac Rehabilitation

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Citation: Cabrera-Aguilera, I.; Ivern, C.; Badosa, N.; Marco, E.; Duran, X.; Mojón, D.; Vicente, M.; Llagostera, M.; Farré, N.; Ruíz-Bustillo, S. Prognostic Utility of a New Risk Stratification Protocol for Secondary Prevention in Patients Attending Cardiac Rehabilitation. *J. Clin. Med.* **2022**, *11*, 1910. https://doi.org/10.3390/jcm11071910

Academic Editor: Gaia Cattadori

Received: 5 February 2022
Accepted: 24 March 2022
Published: 30 March 2022

Abstract: Several risk scores have been used to predict risk after an acute coronary syndrome (ACS), but none of these risk scores include functional class. The aim was to assess the predictive value of risk stratification (RS), including functional class, and how cardiac rehabilitation (CR) changed RS. Two hundred and thirty-eight patients with ACS from an ambispective observational registry were stratified as low (L) and no-low (NL) risk and classified according to exercise compliance; low risk and exercise (L-E), low risk and control (no exercise) (L-C), no-low risk and exercise (NL-E), and no-low risk and control (NL-C). The primary endpoint was cardiac rehospitalization. Multivariable analysis was performed to identify variables independently associated with the primary endpoint. The L group included 56.7% of patients. The primary endpoint was higher in the NL group (18.4% vs. 4.4%, $p < 0.001$). After adjustment for age, sex, diabetes, and exercise in multivariable analysis, HR (95% CI) was 3.83 (1.51–9.68) for cardiac rehospitalization. For RS and exercise, the prognosis varied: the L-E group had a cardiac rehospitalization rate of 2.5% compared to 26.1% in the NL-C group ($p < 0.001$). Completing exercise training was associated with reclassification to low-risk, associated with a better outcome. This easy-to-calculate risk score offers robust prognostic information. No-exercise groups were independently associated with the worst outcomes. Exercise-based CR program changed RS, improving classification and prognosis.

Keywords: acute coronary syndrome; ischemic heart disease; cardiac rehabilitation; exercise training; event-free survival; risk stratification

1. Introduction

Acute coronary syndrome (ACS), one of the main manifestations of ischemic heart disease (IHD), is a leading cause of death worldwide [1]. Many advances in pharmacological and non-pharmacological treatment (e.g., ST-elevation myocardial infarction primary angioplasty initiatives) have been achieved [2]. However, morbidity and mortality remain high. In addition, several risk scores have been used to predict risk in patients

with ACS [3,4]. However, these risk scores are mainly based on in-hospital parameters such as Killip–Kimball class, ST-segment abnormalities, and cardiac biomarkers, among other clinical parameters. Remarkably, none of these risk scores include functional class. In addition, cardiorespiratory capacity estimated by metabolic equivalent (METS) has been consistently associated with prognosis; in ischemic heart disease, patients with poor cardiorespiratory capacity have a much worse prognosis [5,6]. Similarly, left ventricular ejection fraction (LVEF), a well-known prognosis factor, is not always included in the risk scores most used.

Cardiac rehabilitation (CR) after an ACS has a class I indication. However, CR remains widely underused. There are several reasons for this underuse. Patients identify distance, work responsibilities, lack of time, transportation problems, and comorbidities as the most significant barriers to enrolment [7]. Another limitation is logistics, as supervised exercise by physiotherapists, rehabilitation physicians, and cardiologists might not be available in all healthcare settings. To overcome this limitation, there have been several attempts to identify low-risk patients who could perform the unsupervised exercise at home or in a primary care setting. The Spanish Society of Cardiology (SSC) developed a CR protocol stratifying patients based on several parameters. The most relevant parameters were obtained from the echocardiogram and the exercise stress test during hospitalization or after hospital discharge [8]. Patients were categorized as low, mid, and high risk according to this classification. However, the SSC-EXCELENTE cardiac rehabilitation committee later proposed classifying patients only as low (L) or no-low risk (NL). This classification is consistent with that of other international entities and studies that indicate that all patients who are not at low risk should be considered high risk [9–11]. This stratification helps decide where the patient will perform the exercise training, but, surprisingly, it is unknown whether this simple classification can help identify patients with a bad prognosis.

Finally, completing the exercise training of a CR program is associated with an improvement in cardiorespiratory capacity measured by an increase in the METS achieved in the exercise stress test and has also been associated with an increase in LVEF [12,13]. Whether the improvements in function class and LVEF lead to a change in the low vs. no-low risk stratification (RS) and prognosis is substantially unknown.

Hence, the study aimed to assess whether an easy-to-calculate RS could identify patients with a worse prognosis, and how exercise-based CR changed this stratification.

2. Materials and Methods

2.1. Study Design, Population and Study Variables

After an ACS, all patients from the Hospital del Mar reference area are referred to the cardiac rehabilitation unit. From November 2016 to September 2019, 497 were assessed at the cardiac rehabilitation unit and included in the Ambispective Risk Optimization—Acute Coronary Syndrome (Risk-Op-ACS) registry (ClinicalTrials.gov Identifier: NCT03619395). We included patients with ST-elevation acute myocardial infarction (STEMI), non-ST-elevation acute myocardial infarction (non-STEMI), and unstable angina (UA). The diagnosis was made following the European Society of Cardiology guidelines [14,15]. The main exclusion criteria in the CR unit were patients from other health areas, those with a severe language barrier, or patients who refused to participate. For the present study, we only included patients with an assessment of cardiorespiratory capacity by exercise stress testing both at baseline and at the end of the rehabilitation program [16]. Thus, the final study sample included 238 patients.

We collected baseline demographic and clinical data and follow-up events. Follow-up was performed by directly contacting patients or relatives or reviewing medical records.

2.2. Cardiac Rehabilitation Program

The cardiac rehabilitation unit at the Hospital del Mar is an interdisciplinary program that combines interventions performed by cardiologists, nurses, rehabilitation physicians, physiotherapists, and psychiatrists. Detailed information on the unit's characteristics has

been previously described [16]. Briefly, all patients with an ACS receive education by specialized nurses on healthy habits during the ACS hospitalization, at 3 and 12 months after inclusion. Patients attend weekly group sessions with healthcare professionals to reinforce their health education. Finally, all patients are referred to participate in the exercise training component. Cardiorespiratory fitness is assessed at enrollment by a treadmill stress test. According to the patients' characteristics, treadmill stress test and RS, rehabilitation physicians prescribe the setting, level, and type of exercise recommended to each patient. The ET intervention consists of 25 one-hour sessions, five times per week for five weeks. After five-minute warm-up and conditioning, each session begins with thirty minutes of exercise on a cycle ergometer at 80% of effort assessed by cardiorespiratory capacity test followed by twenty minutes of strength and resistance training of both upper and lower extremities with a load of 10 repetitions maximum (10 RM) and ending with a period of five minutes cool-down. The workload progression is adjusted weekly according to the patient's tolerance by the Borg perceived effort scale. Sessions are supervised by an expert physiotherapist, using continuous heart rate and pulse oximetry monitoring. Once the patients complete the 25 sessions, a treadmill stress test is carried out to re-evaluate cardiorespiratory functional status. In patients with reduced baseline LVEF, an echocardiogram is repeated during follow-up at the discretion of their treating cardiologist.

2.3. Cardiac Risk Stratification Process

All patients were stratified according to the risk level score developed by the Spanish Society of Cardiology (SSC) and the recommendation of the SSC-EXCELENTE committee of cardiac rehabilitation [8,9]. Patients in the no-low group had one or more of these parameters: cardiorespiratory capacity <7 METs, angina during the stress test, ST depression >2 mm with heart rate <135 bpm, hypotensive response to exercise or malignant ventricular arrhythmias, reversible wall defects with stress thallium, reinfarction, residual ischemia, depression/anxiety, frailty, history of decompensated heart failure during ACS admission, and LVEF <49%. Patients in the low group comprised all the other patients. The main goal of this classification is to identify low-risk patients who can safely complete the ET component of the cardiac rehabilitation in a setting other than the hospital and, thus, make ET more accessible to all the patients who may benefit from it.

Some patients did not complete the exercise training program for several reasons and were considered the control group. Therefore, we classified the patients into four groups according to exercise compliance and risk stratification: low risk and exercise (L-E), low risk and no exercise (control) (L-C), no-low risk and exercise (NL-E), and no-low risk and no exercise (control) (NL-C).

2.4. Aims and Endpoint

The study's primary aim was to evaluate whether a low and no-low risk stratification can help predict outcomes in patients with a recent ACS. The primary endpoint was cardiac rehospitalization. Cardiac rehospitalization was defined as any cardiac event that required hospital admission for more than 24 h and included: arrhythmias, heart failure, SCA, and unplanned coronary revascularization.

The secondary endpoints were to evaluate whether this risk stratification is modified by participating in exercise training (ET) and to assess whether changes in the group risk classification over time affect prognosis. Finally, we evaluated whether a low and no-low risk stratification was associated with the primary endpoint's individual endpoints.

2.5. Ethics

The study was designed in compliance with the ethical principles set forth by the Declaration of Helsinki. The data included in this study incorporated both data from a prospective and retrospective registry. The prospective registry was carried out from July 2018 to September 2019. The Ethics Committee of the Hospital del Mar (Parc de Salut Mar) approved the study (N° 2018/7896/I), and all patients provided written informed

consent. To increase the sample size, and given that the same protocol had been carried out before, the Ethics Committee approved including patients from November 2016 to June 2018 retrospectively. It waived the need for written informed consent in this group.

2.6. Statistics

Data for continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) based on normality distribution assessed by Kolmogorov–Smirnov test or Shapiro–Wilk test for smaller groups. Categorical variables were expressed as numbers (n) and percentages (%). Differences in baseline characteristics and risk variation between groups previously defined by risk stratification were tested using Chi-square, Student's *t*, or Mann–Whitney U test as needed. For baseline characteristics between groups previously defined by exercise compliance and risk stratification, one-way analysis of variance or Kruskal–Wallis tests were used. Minimal detectable change was calculated following previous studies [17]. Univariable and multivariable analyses were performed using the Cox proportional hazard regression model to examine the association between risk groups and cardiac rehospitalization. Variables with an overall significance value of $p < 0.05$ were entered for multiple Cox regression analysis to identify the strongest predictors for event-free survival. We also included sex, as it is a well-known risk factor. The model was adjusted for potential confounders selected by stepwise forward inclusion, among patient characteristics previously defined.

The number of events per degree of freedom was fairly small, and below the rule of thumb established at 10 events per variable. However, the convenience of this rule of thumb has been largely discussed in the literature in recent years [18]. The proportional hazard assumption, checked by examining residuals (for overall model and variable by variable), was not violated. The log-rank test was performed to compare Kaplan–Meier survival curves. Differences from baseline to follow-up in RS were evaluated using the McNemar test. All analysis was performed using IBM SPSS Statistics v25 (Armonk, NY, USA) and GraphPad Prism 8.0 (San Diego, CA, USA). For all tests, $p < 0.05$ was considered as statistically significant.

3. Results

Baseline clinical characteristics of the study population divided by risk groups are summarized in Table 1. In brief, most participants were middle-aged men admitted due to STEMI and who had a one-vessel disease and preserved ejection fraction. Overall, 56.7% of the patients were in the low-risk group. The main differences between groups were age (59.3 years in the low risk vs. 63.3 years in the no-low risk, $p = 0.006$), ejection fraction (51 vs. 60%, respectively, $p < 0.001$), diabetes mellitus (15.6 vs. 27.2%, $p = 0.028$) and glycated hemoglobin levels (5.6 vs. 5.7%, $p = 0.007$). Supplementary Table S1 shows the difference between patients included in this study and patients who were not.

The median (interquartile range) absolute METs gained in the whole cohort who completed the ET was 1.0 (0.2–2.0). As summarized in Table 2, METs achieved were higher in the low-risk group at baseline and follow-up. Patients in the no-low risk group significantly increased the METs gained (Table 2).

The median follow-up was 31 (23–39) months. Tables 2 and 3 show that the primary endpoint of cardiac rehospitalization was higher in the no-low risk group (18.4% vs. 4.4%, $p < 0.001$, univariable hazard ratio (HR) (95% confidence interval (CI): 4.32 (1.73–10.82)). In multivariable analysis, after adjustment for age, sex, diabetes mellitus, and the completion of the exercise training, the HR (95% CI) was 3.83 (1.51–9.68) (Table 3). Figure 1A shows the Kaplan–Meier survival curve with a better prognosis in the low-risk group. Supplementary Table S2 shows the difference between patients with cardiac rehospitalization and patients without. Interestingly, the only differences were the presence of hyperlipidemia, anemia, and the number of coronary arteries affected.

Table 1. Baseline characteristics according to the low and no-low groups.

Characteristic/Variable	L (*n* = 135)	NL (*n* = 103)	*p*-Value
Anthropometric			
Age (years)	59.3 ± 10.2	63.3 ± 10.6	0.006
Women	19 (14.1)	17 (16.5)	0.604
BMI (kg/m^2)	27.3 (24.7–29.3)	26.9 (24.9–30.5)	0.631
Risk factors and comorbidities			
Hypertension	64 (47.4)	56 (54.4)	0.287
Hyperlipidemia	92 (68.1)	65 (63.1)	0.416
Diabetes mellitus	21 (15.6)	28 (27.2)	0.028
Current smoker	59 (43.7)	33 (32.0)	
Previous smoker > 1 year	42 (31.1)	35 (34.0)	0.288
Previous smoker < 1 year	5 (3.7)	5 (4.9)	
COPD	5 (3.7)	10 (9.7)	0.059
Cerebrovascular disease	3 (2.2)	7 (6.8)	0.081
Peripheral vascular disease	6 (4.4)	3 (2.9)	0.539
Anemia	13 (9.3)	13 (12.6)	0.464
Chronic kidney disease	3 (2.2)	5 (4.9)	0.264
Diagnostics			
STEMI	60 (40.4)	46 (44.7)	
NSTEMI	54 (40.0)	37 (35.9)	0.684
Unstable angina	21 (15.6)	20 (19.4)	
Previous ACS-MI	17 (12.6)	18 (17.5)	0.292
One vessel disease	84 (62.2)	51 (49.5)	
Two vessels disease	29 (21.5)	26 (25.2)	0.133
Three vessels disease	17 (16.2)	24 (23.3)	
Ejection fraction (%)	60 (55.0–63.5)	51 (43.5–60.0)	<0.001
Exercise testing			
METs	10.3 (9.1–12.4)	8.3 (6.6–9.8)	<0.001
Maximum predicted heart rate (%)	81 (73.0–89.0)	79 (67.0–87.0)	0.073
Peak systolic blood pressure (mmHg)	154 (142–173)	148 (132–165)	0.055
Blood test			
Glucose, mg/dL	104 (94–124)	116 (99–151)	0.005
Glycated hemoglobin, %	5.6 (5.3–5.8)	5.7 (5.4–6.7)	0.007
LDLc, mg/dL	117 (90–136)	107(81–136)	0.323

Data are mean ± SD, median (IQR), or numbers (n) and percentages (%). L, Low group; NL, No-low group; ACS, acute coronary syndrome-myocardial infarction; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; METs, metabolic equivalent; MI, myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.

Table 2. Outcomes according to the low and no-low groups.

Outcomes	L (*n* = 135)	NL (*n* = 103)	*p*-Value
Follow-up METs	11.8 (9.8–13.3)	9.8 (7.9–11.4)	<0.001
Relative increase in METs (%)	7.6 (0.0–17.4)	12.3 (2.6–27.4)	0.019
Absolute increase in METs	0.8 (0.0–1.9)	1.1 (0.2–2.1)	0.173
All causes re-admission	27 (20.0)	39 (37.9)	0.002
All causes death	0 (0.0)	2 (1.9)	0.104
Cardiac rehospitalization	6 (4.4)	19 (18.4)	<0.001
Heart failure	1 (0.7)	3 (2.9)	0.197
Arrythmias	2 (1.5)	10 (9.7)	0.004
Revascularization	4 (3.0)	9 (8.7)	0.052
New ACS	0 (0.0)	7 (6.8)	0.002

Data are median (IQR) or numbers (n) and percentages (%). L, Low group; NL, No-low group; ACS, acute coronary syndrome-myocardial infarction; METs, metabolic equivalent.

Table 3. Univariable and multivariable Cox regression analyses for cardiac rehospitalization.

	Univariable HR (95% CI)	*p*-Value	Adjusted HR (95% CI)	*p*-Value
No-low risk group	4.32 (1.73–10.82)	0.002	3.83 (1.51–9.68)	0.005
No-exercise group	3.15 (1.39–7.15)	0.006	2.52 (1.10–5.78)	0.021
Age (years)	1.03 (0.99–1.07)	0.103	-	
Men	1.22 (0.36–4.10)	0.745	-	
Mellitus diabetes	1.40 (0.47–4.02)	0.556	-	

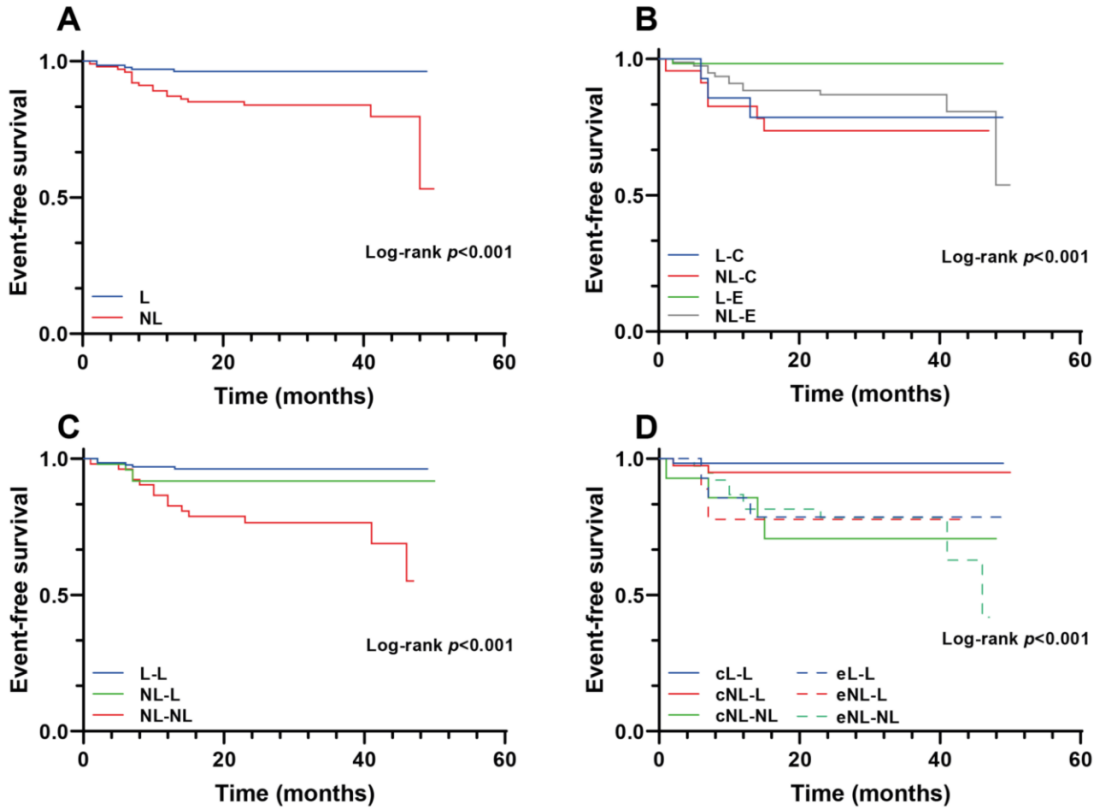

Figure 1. Kaplan–Meier curves according to the different risk groups. Panel (**A**): Global risk stratification. Panel (**B**): Exercise compliance classification. Panel (**C**): Rehabilitation risk stratification variation. Panel (**D**): Rehabilitation exercise compliance risk stratification variation. L, Low-risk group; NL, No-low risk group; C, Control; E, exercise; L-L, low to low risk; NL-L, No-low to low risk; NL-NL, No-low to no-low risk.

Table 4 shows the differences in baseline characteristics and prognosis according to the risk group and the completion of the exercise training. Only 15.6% were in the control group, and of those, 37.8% were in the low-risk group, and 62.2% were in the no-low group. In the exercise group, 60% were in the low-risk group and 39.8% in the no-low risk group. Interestingly, the only differences between groups were age and glycated hemoglobin. Outcomes were different (Table 5 and Figure 1B), with cardiac rehospitalization of 2.5% in the low-risk exercise group compared to 26.1% in the no-low no-exercise (control) group ($p < 0.001$).

Table 4. Baseline characteristics according to risk stratification and exercise training completion.

Characteristic/Variable	L-C (*n* = 14)	NL-C (*n* = 23)	L-E (*n* = 121)	NL-E (*n* = 80)	*p*-Value
Anthropometric					
Age (years)	61.4 ± 9.3	65.5 ± 12.1	59.8 ± 9.7	62.1 ± 10.1	0.005
Women	3 (21.4)	5 (21.7)	16 (13.2)	12 (15.0)	0.669
BMI (kg/m^2)	26.7 (24.0–27.7)	28.4 (23.5–31.2)	27.3 (24.7–29.3)	26.7 (24.8–30.5)	0.830
Risk factors and comorbidities					
Hypertension	6 (42.9)	16 (69.6)	58 (47.9)	40 (50.0)	0.262
Hyperlipidemia	8 (57.1)	13 (56.5)	84(69.4)	52 (65.0)	0.557
Diabetes mellitus	1 (7.1)	8 (34.8)	20 (16.5)	20 (25.0)	0.088
Current smoker	9 (64.3)	7 (30.4)	50 (41.3)	26 (32.5)	
Previous smoker > 1 year	3 (21.4)	6 (26.1)	39 (32.2)	29 (36.3)	0.478
Previous smoker < 1 year	0 (0.0)	1 (4.3)	5 (4.1)	4 (5.0)	
COPD	1 (7.1)	2 (8.7)	4 (3.3)	8 (10.0)	0.269
Cerebrovascular disease	0 (0.0)	2 (8.7)	3 (2.5)	5 (6.3)	0.322
Peripheral vascular disease	1 (7.1)	0 (0.0)	5 (4.1)	3 (3.8)	0.710
Anemia	3 (21.4)	4 (17.4)	10 (8.3)	9 (11.3)	0.325
Chronic kidney disease	0 (0.0)	2 (8.7)	3 (2.5)	3 (3.8)	0.419
CV family history	3 (21.4)	4 (17.4)	26 (21.5)	12 (15.0)	0.702
Sudden death family history	0 (0.0)	1 (4.3)	6 (5.0)	1 (1.3)	0.457
Diagnostics					
STEMI	4 (28.6)	9 (39.1)	56 (46.3)	37 (46.3)	
NSTEMI	7 (50.0)	8 (34.8)	47 (38.8)	29 (36.3)	0.765
Unstable Angina	3 (21.4)	6 (26.1)	18 (14.9)	14 (17.5)	
Previous ACS-MI	3 (21.4)	7 (30.4)	14 (11.6)	11 (13.8)	0.109
One vessel disease	8 (57.1)	7 (30.4)	76 (62.8)	44 (55.0)	
Two vessels disease	4 (28.6)	7 (30.4)	25 (20.7)	19 (23.8)	0.228
Three vessels disease	2 (14.3)	9 (39.1)	15 (12.4)	15 (18.8)	
Ejection fraction (%)	62 (55.5–63.5)	58 (51.0–60.0)	60 (56.0–64.0)	51 (41.0–60.0)	<0.001
Exercise testing					
METs	9.8 (8.5–11.8)	6.8 (6.2–7.8)	10.3 (9.3–12.6)	8.5 (6.7–10.0)	<0.001
Maximum predicted heart rate (%)	80 (72.0–83.5)	82 (71.0–87.0)	81 (74.0–89.0)	76 (65.5–85.5)	0.088
Peak systolic blood pressure (mmHg)	155 (139–180)	160 (133–183)	154 (142–172)	147 (132–161.5)	0.103
Blood test					
Glucose, mg/dL	101 (87–112)	126 (106–190)	105 (95–124)	116 (97–151)	0.032
Glycated hemoglobin, %	5.6 (5.3–5.7)	6.7 (5.7–8.3)	5.6 (5.4–5.9)	5.6 (5.4–6.5)	0.016
LDL, mg/dL	115 (89–129)	102 (69–110)	118 (93–140)	111 (84–139)	0.127
Outcomes					
Follow-up METs	10.8 (8.3–12.2)	8.1 (6.0–10.8)	12.0 (9.8–13.3)	9.8 (8.3–11.5)	<0.001
Relative increase in METs (%)	4.5 (−4.3–16.7)	6.5 (−3.2–27.9)	7.7 (0.8–17.6)	14.3 (3.2–26.8)	0.039
Absolute increase in METs	0.3 (−0.4–1.0)	0.5 (−0.2–1.2)	0.9 (0.0–1.9)	1.3 (0.3–2.3)	0.119
Cardiac rehospitalization	3 (21.4)	6 (26.1)	3 (2.5)	13 (16.3)	<0.001
All causes readmission	7 (50.0)	12 (52.2)	20 (16.5)	27 (33.8)	<0.001
All causes death	0 (0.0)	1 (4.3)	0 (0.0)	1 (1.3)	0.195
Revascularization	3 (21.4)	3 (13.0)	1 (0.8)	6 (7.5)	0.002
New ACS	0 (0.0)	2 (8.7)	0 (0.0)	5 (6.3)	0.020

Data are mean ± SD, median (IQR), or numbers (n) and percentages (%). L-C, Low risk and control group; NL-C, No-low risk and control group; L-E, Low risk and exercise group; NL-E, No-low risk and exercise group; ACS, acute coronary syndrome-myocardial infarction; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; METs, metabolic equivalent; MI, myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.

Table 5. Outcomes according to risk stratification and exercise training completion.

Outcomes	L-C (n = 14)	NL-C (n = 23)	L-E (n = 121)	NL-E (n = 80)	p-Value
Follow-up METs	10.8 (8.3–12.2)	8.1 (6.0–10.8)	12.0 (9.8–13.3)	9.8 (8.3–11.5)	<0.001
Relative increase in METs (%)	4.5 (−4.3–16.7)	6.5 (−3.2–27.9)	7.7 (0.8–17.6)	14.3 (3.2–26.8)	0.039
Absolute increase in METs	0.3 (−0.4–1.0)	0.5 (−0.2–1.2)	0.9 (0.0–1.9)	1.3 (0.3–2.3)	0.119
All causes readmission	7 (50.0)	12 (52.2)	20 (16.5)	27 (33.8)	<0.001
All causes death	0 (0.0)	1 (4.3)	0 (0.0)	1 (1.3)	0.195
Cardiac rehospitalization	3 (21.4)	6 (26.1)	3 (2.5)	13 (16.3)	<0.001
Heart failure	1 (7.1)	1 (4.3)	0 (0.0)	2 (2.5)	0.116
Arrythmias	0 (0.0)	3 (13.0)	2 (1.7)	7 (8.8)	0.029
Revascularization	3 (21.4)	3 (13.0)	1 (0.8)	6 (7.5)	0.002
New ACS	0 (0.0)	2 (8.7)	0 (0.0)	5 (6.3)	0.020

Data are median (IQR) or numbers (n) and percentages (%). L-C, Low risk and control group; NL-C, No-low risk and control group; L-E, Low risk and exercise group; NL-E, No-low risk and exercise group; ACS, Acute coronary syndrome-myocardial infarction; METs, metabolic equivalent.

Figure 2 shows the change in the risk classification after the training exercise (or repeated treadmill exercise stress test in the control group). Significant increase (from 56.7% to 77.3%, $p < 0.001$) and decrease (from 43.3% to 22.7%, $p < 0.001$) were observed in low and no-low groups respectively. Participation in the exercise training was associated with a significantly higher proportion of patients classified as low risk (McNemar test, $p < 0.001$). All patients in the low-risk group (both in the control and exercise group) remained at low risk. Of patients in the no-low risk group, 60.9% remained in the no-low risk group in the control group, compared with 50% of patients who remained in the no-low risk group in the exercise group. Figure 1C shows that the patients who remained in the no-low group had a worse prognosis. However, patients whose functional class improved enough to be in the low-risk group had a similar outcome to those who were always in the low-risk group. Figure 1D shows that patients in the exercise group who changed to low risk had an excellent prognosis. Those who remained in the no-low risk group had a prognosis similar to the control group.

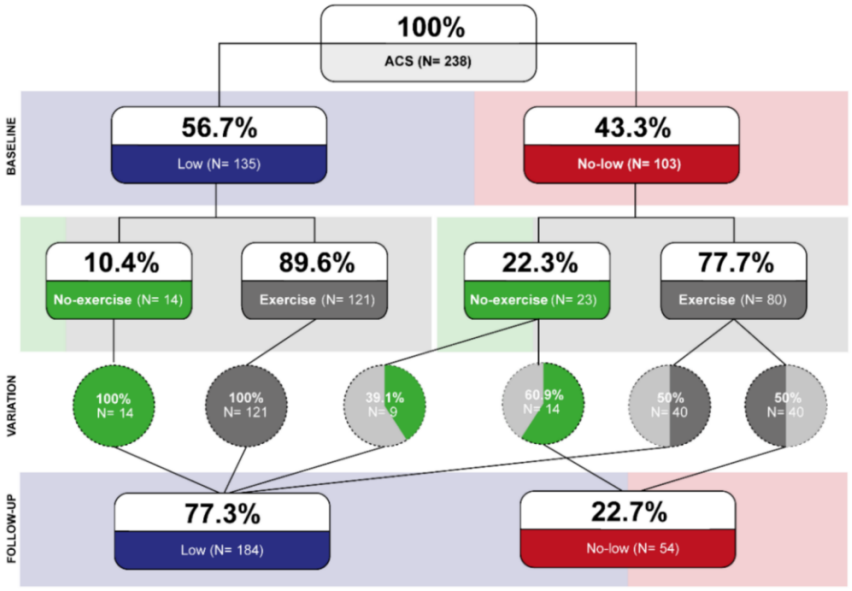

Figure 2. Risk stratification variation.

4. Discussion

One of the most relevant findings from this study was that 56.7% of patients who had been admitted due to an ACS were low-risk according to an easy-to-calculate score that included cardiorespiratory capacity and left ventricular ejection fraction. Cardiac rehospitalization in the no-low risk group was significantly higher than in the low-risk group (HR 3.83 (95 CI 1.51–9.68)). The completion of the exercise program was also independently associated with a better prognosis, and 50% of the patients in the no-low risk group who completed it became low-risk.

Baseline characteristics did not differentiate patients in the low- and no-low risk groups. The only differences were age and diabetes, but these variables were not strikingly different. Indeed, the mean age was 61.0 ± 10.5, and there was only a 4-year difference between groups. In some of the most used risk scores, this difference would not have made significant changes in stratification. Indeed, the GRACE and TIMI score considers age a risk when the patient is older than 65 years [19–21]. Diabetes is a significant risk factor among patients suffering from a myocardial infarction. It is included in the GRACE score [22], and glycated hemoglobin after an ACS might also predict future events [23,24]. In our study, diabetes was more prevalent in the no-low risk group (27.2 vs. 45.6%, $p = 0.028$). Although glycated hemoglobin was statistically higher in the no-low risk group, the differences were clinically not relevant (5.7% (5.4–6.7) vs. 5.6% (5.3–5.8), $p = 0.007$). Compared to patients who did not decide to participate in the ET, patients in the present study were younger, less frequently female, and with less past medical history of hypertension, diabetes mellitus, chronic kidney disease, anemia, and past acute coronary syndrome or acute coronary syndrome myocardial infarction. These differences are consistent with previous literature [25].

As expected, due to the variables included in the stratification protocol, LVEF and cardiorespiratory capacity differed between both groups. However, it is worth noting that the median LVEF was >50% in both groups. Interestingly, although patients in the low-risk group had higher METs (10.3 vs. 8.3, $p < 0.001$), both groups had good cardiorespiratory capacity. Several studies have shown that METs vary with age, but patients in their 50 s and 60 s have a cardiorespiratory capacity of 6 to 10 METs [26,27]. Our cohort comprised middle-aged males with a high prevalence of risk factors but a relatively low prevalence of comorbidities and previous ACS. Moreover, most patients had one-vessel disease and presented with STEMI. Therefore, it is likely that this cohort was reasonable fit before the ACS. After completing the ET, there was an increase in the relative increase and total METs achieved in the treadmill stress test. Although not significant, there was also an increase in the absolute increase of METs achieved by the four groups (0.3 to 1.3 METS in the low-risk control and no-low risk and ET). This absolute increase is similar to the 0.52 to 1.55 METs increase described in other studies and meta-analyses [28]. It also compares favorably to the 0.21 minimal detectable change expected.

The low-risk patients had a cardiac rehospitalization rate much lower than the no-low risk group (4.4% vs. 18.4%, $p < 0.001$). After multivariable analysis, patients in the no-low risk group had a worse prognosis with an HR 3.83 (95% CI 1.51–9.68) for the primary endpoint. Other risk scores have shown that prognosis after an ACS worsens with increased risk. Risk stratification of the GRACE score indicated that the mortality risk of the intermediate-risk and high-risk groups was higher with an HR 3.23 (1.59–6.55) for the intermediate-risk group and HR 15.31 (4.43–51.62) for the highest risk group. Similar results were observed with MACCE risk [29]. Still, different endpoints, follow-up periods, and baseline characteristics can explain the differences in outcomes with our results, especially in the high-risk group. Hospitalization due to heart failure was infrequent in our cohort (two patients, 1.7%) and was numerically much higher in the no-low risk group. Five percent of the patients experienced hospitalization due to an arrhythmic event, which was more frequent in the no-low risk group. This finding is consistent with previous reports that showed benefits of CR in patients with arrhythmia [30].

Finally, this study showed that all patients initially classified in the low-risk group remained in this group. However, completion of the exercise training was associated with reclassification from the no-low to low-risk group more frequently than in the control group. The no-low risk group who completed the exercise training had the most significant improvement with a relative increase of 14.3% in the METS achieved in the treadmill stress test. This reclassification was associated with a better outcome. Although several studies and meta-analyses have shown that exercise training is associated with a better prognosis [31–33], few studies have analyzed whether the change in risk categories is associated with prognosis. The proposed risk score could identify no-low risk patients soon after an ACS and add further prognostic information after an exercise training program.

The main limitation of this study is that the results might not apply to other settings as a single-center observational study. Some of the patients were included retrospectively, which might lead to bias. However, we believe that the risk of bias is low since all patients followed the same protocol and that the information was documented in the medical record in a structured way

5. Conclusions

Given that this easy-to-calculate routine risk stratification method offers prognostic information, it should be used in all patients after an ACS. Low-risk patients had an excellent prognosis compared to the no-low risk group. Exercise-based CR program showed the ability to change risk stratification, improving functional classification and prognosis of these patients who initially belonged to the no-low risk and ended as low risk. Therefore, this risk stratification score could identify patients suitable for exercise training in an unsupervised setting and identify low-risk patients with excellent prognosis at follow-up.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11071910/s1, Table S1: Baseline characteristics between patients who attended cardiac rehabilitation and the whole cohort of ambispective registry; Table S2: Baseline characteristics between patients who had cardiac readmission and those who did not.

Author Contributions: Conceptualization, S.R.-B., N.B., I.C.-A. and N.F.; methodology, S.R.-B. and N.F.; software, I.C.-A.; validation, S.R.-B., N.F. and I.C.-A.; formal analysis, I.C.-A., X.D. and N.F.; investigation, N.B., C.I., M.V., M.L., D.M. and I.C.-A.; data curation, I.C.-A.; writing—original draft preparation, I.C.-A. and N.F.; writing—review and editing, E.M. and S.R.-B.; supervision, N.F. All authors have read and agreed to the published version of the manuscript.

Funding: I. Cabrera-Aguilera is supported by ANID-PFCHA—Chilean Doctorate Fellowship 2017; Grant No. 72180089.

Institutional Review Board Statement: The study was conducted according to the guidelines and designed in compliance with the ethical principles set forth by the Declaration of Helsinki and approved by the Ethics Committee of the Hospital del Mar (Parc de Salut Mar) (N° 2018/7896/I).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the prospective study. Patient consent was waived for patients included retrospectively.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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Table S1. Baseline characteristics between patients who attended cardiac rehabilitation and the whole cohort of ambispective registry.

Characteristic/Variable	Total (*n* = 497)	Included (*n* = 238)	Not-Included (*n* = 259)	*p* Value
Anthropometric				
Age (years)	62.0 (54–72)	61.0 (53–69)	64.0 (55–75)	0.006
Women	93 (18.7)	36 (15.1)	57 (22.0)	0.049
BMI (Kg/m2)	27.1(24.8–30.1)	27.2(24.8–29.7)	27.0(24.6–30.4)	0.945
Risk factors and comorbidities				
Hypertension	281 (56.5)	120 (50.4)	161 (62.2)	0.008
Hyperlipidemia	324 (65.2)	157 (66.0)	167 (64.5)	0.728
Diabetes mellitus	143 (28.8)	49 (20.6)	94 (36.3)	<0.001
Current smoker	205 (41.2)	92 (38.7)	113 (43.6)	
Previous smoker > 1 year	146 (29.4)	77 (32.4)	69 (26.6)	0.529
Previous smoker < 1 year	20 (4.0)	10 (4.2)	10 (3.9)	
COPD	40 (8.0)	15 (6.3)	25 (9.7)	0.170
Cerebrovascular disease	23 (4.6)	10 (4.2)	13 (5.0)	0.665
Peripheral vascular disease	39 (7.8)	9 (3.8)	30 (11.6)	0.001
Anemia	87 (17.5)	26 (10.9)	61 (23.6)	<0.001
Chronic kidney disease	29 (5.8)	8 (3.4)	21 (8.1)	0.024
CV family history	92 (18.5)	45 (18.9)	47 (18.1)	0.827
Sudden death family history	11 (2.2)	8 (3.4)	3 (1.2)	0.095
Diagnostics				
STEMI	220 (44.3)	106 (44.5)	114 (44.0)	0.131
NSTEMI	174 (35.0)	91 (38.2)	83 (32.0)	0.131
Unstable Angina	103 (20.7)	41 (17.2)	62 (23.9)	
Previous ACS-MI	99 (19.9)	35 (14.7)	64 (24.7)	0.005
One vessel disease	263 (52.9)	135 (56.7)	128 (49.4)	
Two vessels disease	126 (25.4)	55 (23.1)	71 (27.4)	0.412
Three vessels disease	87 (17.5)	41 (17.2)	46 (17.8)	
Ejection fraction (%)	59 (52–62)	58 (52–63)	59 (53–62)	0.184
Blood test				
Glucose, mg/dL	108 (95- 135)	107 (95–131)	109 (95–135)	0.465
Glycated hemoglobin, %	5.7 (5.4–6.5)	5.6 (5.4–6.1)	5.9 (5.4–6.8)	0.001
LDL, mg/dL	109 (78–135)	113 (87–137)	102 (73–125)	0.006

Data are mean ± SD, median (IQR), or numbers (*n*) and percentages (%). ACS, Acute coronary syndrome-myocardial infarction; BMI, Body mass index; COPD, Chronic obstructive pulmonary disease; CV, Cardiovascular; MI, myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.

Table S2. Baseline characteristics between patients who had cardiac readmission and those who did not.

Characteristic/Variable	Cardiac Readmission (n = 25)	Withouth Cardiac Readmission (n = 213)	p Value
Anthropometric			
Age (years)	64 (58–70)	61 (53–69)	0.101
Women	3 (12.0)	33 (16)	0.645
BMI (Kg/m2)	27.6 (24.5–29.6)	27.1 (24.8–29.6)	0.874
Risk factors and comorbidities			
Hypertension	15 (60)	105 (49)	0.311
Hyperlipidemia	11 (44)	146 (69)	0.014
Diabetes mellitus	4 (16)	45 (21)	0.549
Current smoker	4 (16)	88 (41.3)	
Previous smoker > 1 year	13 (52)	64 (30.0)	0.067
Previous smoker < 1 year	1 (4.0)	9 (4.2)	
COPD	3 (12.0)	12 (5.6)	0.215
Cerebrovascular disease	1 (4.0)	9 (4.2)	0.958
Peripheral vascular disease	0 (0.0)	9 (4.2)	0.295
Anemia	6 (24.0)	20 (9.4)	0.027
Chronic kidney disease	2 (8.0)	6 (2.8)	0.174
CV family history	2 (8.0)	43 (20.2)	0.141
Sudden death family history	0 (0.0)	8 (3.8)	0.324
Diagnostics			
STEMI	9 (36.0)	97 (45.5)	
NSTEMI	10 (40.0)	81 (38.0)	0.542
Unstable Angina	6 (24.0)	35 (16.4)	
Previous ACS-MI	6 (24.0)	29 (13.6)	0.165
One vessel disease	8 (32.0)	127 (59.6)	
Two vessels disease	8 (32.0)	47 (22.1)	0.033
Three vessels disease	9 (36.0)	32 (15.0)	
Ejection fraction (%)	57 (51–64)	58 (52–62)	0.268
Blood test			
Glucose, mg/dL	109 (102–156)	106 (95–128)	0.130
Glycated hemoglobin, %	5.7 (5.4–6.5)	5.6 (5.4–6.0)	0.414
LDL, mg/dL	97 (71–121)	113 (88–139)	0.063

Data are mean ± SD, median (IQR), or numbers (n) and percentages (%). ACS, Acute coronary syndrome-myocardial infarction; BMI, Body mass index; COPD, Chronic obstructive pulmonary disease; CV, Cardiovascular; MI, myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.

Chapter VII:
RESULTS SUMMARY

Three different research articles have been included in this Thesis. Their main aim was to contribute to the knowledge of muscular dysfunction and cardiac rehabilitation, from translational animal models to clinical repercussions and impact in CVD. On the one hand, testing the hypothesis that the widespread rodent model of isoproterenol-induced HF results in increased diaphragmatic contractility. On the other hand, comparing clinical outcomes and assessing the predictive value of RS between patients with different compliance and initial attitude for accept or reject the exercise component of cardiac rehabilitation.

3 From muscular physiology in animals

The first study focuses on characterizing and analyzing the muscular function in the diaphragm of an animal model not previously described in the literature. At baseline and 30 days after treatment with isoproterenol or saline, echocardiography was measured following a standard protocol. Isoproterenol mice showed echocardiographic indices characteristic of HF at the end-point. An increase in structural parameters (LVEDD: 0.54 ± 0.05 mm vs 0.17 ± 0.10 mm, p=0.008 and LVESD: 0.59 ± 0.05 mm vs 0.13 ± 0.11 mm, p=0.019) and a significant decrease in functional parameters (LVEF: 8.50 ± 1.37 % vs 0.80 ± 1.75 %, p=0.003 and FS: 6.20 ± 0.98 % vs 0.70 ± 1.45 %, p=0.006) as compared with healthy controls, respectively. Confirming heart hypertrophy and impaired cardiac function. This, along pulmonary edema, assessed in each lung as the wet/dry (W/D) weight ratio which increased in the HF group (5.93 ± 0.32 vs 4.58 ± 0.19, p=0.004), confirmed the CVD model, especially HF.

Diaphragmatic echography was performed immediately after echocardiography, following a standard protocol for non-invasively measuring diaphragm function in mice. M-mode was used to measure the diaphragm movement during regular breathing cycles, detecting the diaphragm's contraction (positive deflection) and relaxed state (negative deflection). The diaphragmatic excursion, which was quantified as the amplitude of movement between the lowest and peak point of the contraction, was calculated as the change from baseline measure and was significantly higher in HF mice as compared with healthy animals (0.55 ± 0.62 mm vs 0.19 ± 0.34 mm, p<0.01).

Ex-vivo assessment of diaphragmatic contractile function was carried out using an isolated muscle test system. During the entire experimental setting, muscle tissue had the physiological conditions to maintain homeostasis and correct tissue function. In the same direction as *in-vivo* measurement, isolated muscle test showed increased diaphragmatic

contractility in isoproterenol-induced HF mice respect healthy control in replication of all physiological situations of muscle contraction measures. Maximum force observed with tetanic contraction (208.75 ± 30.79 mN/mm^2 vs 135.92 ± 69.05 mN/mm^2, $p<0.05$) and when elicit a peak specific twitch force in response to a single supramaximal stimulus, diaphragm shown the same tendency, with increased force production respect healthy control mice.

In summary, diaphragmatic contractility increased by ≈50% in both peak specific twitch and tetanic forces. Fatigue resistance experiments showed a significant decrease in time to half initial force in HF compared with healthy control mice and a significant increase in the same group in the strength decrement index (SDI), indicating increased fatigue in HF diaphragms.

Finally, myosin gene expression of specific muscle fiber type (muscle fiber type MyHC-I correlates with *Myh6*, MyHC-IIa with *Myh2*, MyHC-IId/x with *Myh1* and MyHC-IIb with *Myh4*). Gene expression of *Myh6*, *Myh2*, and *Myh1* at the diaphragm samples showed no significant differences when comparing HF and healthy mice. By contrast, *Myh4* expression showed a significant 2-fold increase in HF animals, supporting a greater predominance of type IIb fibers over other types of muscle fibers in diaphragm samples of isoproterenol-induced HF animals.

4 To prognosis impact in patients

Approaching of clinical repercussions and impact of muscle dysfunction in CVD, we compared clinical outcomes and measured the predictive value of RS between patients with different compliance and initial attitude for accept or reject exercise component of cardiac rehabilitation.

The second article aimed to assess whether there were epidemiological differences between patients referred for EB-CRP according to two variables: exercise compliance (EC) and initial attitude toward ET. This study analyzed the reasons for rejecting or not completing ET and explored the prognosis impact of each group, including prognostic analyses of a sub-group of patients according to initial attitude. A total of 497 patients diagnosed with ACS from an ambispective observational registry study carried out at the Hospital del Mar were included and classified into three groups: those who completed the ET (A-T), those who outrightly rejected ET (R-NT), and those who accepted but did not complete ET (A-NT). Most patients were men diagnosed with STEMI, 1-vessel disease, and preserved ejection fraction.

Patients who completed ET (A-T) were younger and had fewer comorbidities. Patients in R-NT and A-NT groups had similar baseline characteristics.

Initially, 70% of patients accepted participating in the ET, but only 50.5% completed it and 28% of this group did not complete the ET component for various reasons. The main reason for outrightly rejecting to participate in or not completing the ET component was rejection-reason unknown, functional problems and or work/schedule incompatibility. Notably, none of the A-T patients experienced a CV complication while exercising, confirming that ET was safe in this group of patients.

Prognosis impact in patients using a frequently-used primary composite endpoint of all-cause mortality, hospitalization for ACS, or need for revascularization during 31 months median follow-up showed that 12% of all patients had the composite outcome. From these, the A-T group showed an excellent prognosis. In this group, the mortality rate was < 1%. The prognosis in the A-NT and R-NT groups was similar and worse than in the A-T group. After adjustment for age, sex, anemia, hypertension, diabetes mellitus, renal disease, previous ACS, peripheral vascular disease, type of ACS, and ET compliance, the only variables that remained statistically significant with the composite endpoint on multivariable Cox regression analysis were ET compliance [A-NT, HR 5.6 (95% CI 2.75–13.05); R-NT, HR 4.32 (2.00–9.29)], previous ACS and anemia.

The third study included in this Thesis focuses on assessing the predictive value of RS between patients with different exercise compliance and evaluating if RS could identify patients with a worse prognosis. Finally, the last aim was to resolve if EB-CRP changed this stratification. From the same previous ambispective observational registry, we only included patients with an assessment of cardiorespiratory capacity by exercise stress testing both at baseline and at the end of the rehabilitation program. Thus, finally, we included 238 patients. Most participants were middle-aged men admitted due to STEMI who had a one-vessel disease and preserved ejection fraction.

For several reasons, some patients did not complete the exercise training program and were considered the control group. Therefore, we classified the patients into four groups according to exercise compliance and risk stratification: low risk and exercise (L-E), low risk and no exercise (control) (L-C), no-low risk and exercise (NL-E), and no-low risk and no exercise (control) (NL-C). Overall, 56.7% of the patients were in the low-risk group. The main differences between groups at baseline were age (59.3 years in the low risk vs. 63.3 years

in the no-low risk, p = 0.006), ejection fraction (51 vs. 60%, respectively, p < 0.001), diabetes mellitus (15.6 vs. 27.2%, p = 0.028) and glycated hemoglobin levels (5.6 vs. 5.7%, p = 0.007).

The primary endpoint was cardiac rehospitalization, defined as any cardiac event that required hospital admission for more than 24 h and included: arrhythmias, HF, SCA, and unplanned coronary revascularization and was higher in the no-low risk group (18.4% vs. 4.4%, p < 0.001, univariable hazard ratio (HR) (95% confidence interval (CI): 4.32 (1.73–10.82)). In multivariable analysis, after adjustment for age, sex, diabetes mellitus, and the completion of the exercise training, the HR (95% CI) was 3.83 (1.51–9.68). (Table 3). Kaplan-Meier survival curve shows a better prognosis in the low-risk group.

When we added EC as a variable, 15.6% were in the control group. Of those, 37.8% were in the low-risk group and 62.2% were in the no-low group. In the exercise group, 60% were in the low-risk group and 39.8% in the no-low risk group. Interestingly, the only differences between groups were age and glycated hemoglobin. Outcomes were different, with cardiac rehospitalization of 2.5% in the low-risk exercise group compared to 26.1% in the no-low no-exercise (control) group (p < 0.001).

Changes in the risk classification after the ET showed significant increase (from 56.7% to 77.3%, p < 0.001) and decrease (from 43.3% to 22.7%, p < 0.001) in low and no-low groups, respectively. Participation in the ET was associated with a significantly higher proportion of patients classified as low risk (McNemar test, p < 0.001). All patients in the low-risk group (both in the control and exercise group) remained at low risk. Of patients in the no-low risk group, 60.9% remained in the no-low risk group in the control group, compared with 50% of patients who remained in the no-low risk group in the exercise group.

As expected, patients who remained in the no-low group had a worse prognosis. However, patients whose functional class improved enough to be in the low-risk group had a similar outcome to those who were always in the low-risk group. Patients in the exercise group who changed to low risk had an excellent prognosis. Those who remained in the no-low risk group had a prognosis similar to the control group.

Completing ET was associated with reclassification to low-risk and better outcomes. This easy-to-calculate risk score offers robust prognostic information. No-exercise groups were independently associated with the worst outcomes. Exercise-based CR program changed RS, improving classification and prognosis.

Chapter VIII:
Discussion

5 Global discussion

CVD diseases are very prevalent and a significant worldwide health problem. They reduce the quality of life while prevalence continues rising due to improvements in diagnostic and therapeutic management causing people to live with the consequences of a cardiovascular event for longer time.

The growing interest in improving therapeutic rehabilitation strategies in translational and clinical research has focused on improving health strategies through low-cost, high-impact tools such as exercise training. While the most invasive approaches are mainly explored through animal models, retrospective studies have shown multidomain utility from a clinical approach.

On the one hand, at the basic-translational level, there are animal models of CVD such as the widely used isoproterenol-induced HF that has contributed significantly to the study of novel insights into cardioprotection pathways, pharmacological research, cardiac fibrosis, among others (71,111–114). But, despite the growing interest in muscle research for its anti-inflammatory-secretory capabilities, diaphragmatic muscle function for exploring cardiorespiratory rehabilitation pathways in this mouse model of HF had not been previously described. On the other hand, from a clinical perspective, the impact of muscular dysfunction is multiple but largely preventable and reversible. Surprisingly, in secondary prevention, the challenges focused on improving participation into EB-CRP remains underused despite having a recommentation class 1A in clinical guidelines.

Health strategies provide an adequate and safe response throughout interdisciplinary programs. Improving these programs of secondary prevention detailing adherence strategies and enhancing risk stratification procedures could be valuable in the development of therapeutics. Finally, research that considers new subpopulations across adherence variables and looks for another utility in existing RS protocols could provide relevant information for strategically planning changes to ensure reduction in structural barriers and improvement in participation and adherence.

5.1 Effectiveness of the animal model of CVD

Results reveal that the conventional HF rodent model based on continuous infusion of isoproterenol for 30 days is associated with considerable enhancement of diaphragm contractility when assessed noninvasively as diaphragm excursion (by ≈30%) or when measured *ex vivo* (by ≈50% in both peak specific twitch and tetanic forces). Despite this

model is very effective in mimicking the pathophysiology in both structural (increase 17% in LVEDD and 24% in LVESD) and functional parameters (decay in LVEF by 12% and FS by 18%) and provokes lung edema characterizing HF (increase by 29% in the W/D index), it fails in reproducing the well-known muscular dysfunction that produces weakening of diaphragm in HF patients.

5.2 Echography assessment of diaphragm (dys)function

Regarding the central evaluation of the diaphragm dysfunction, we used *in-vivo* echography and *ex vivo* specific force measurements. Diaphragmatic ultrasound has gained importance in the last years for many reasons. Among its remarkable advantages is the fact that it is non-invasive, does not expose patients/animals to radiation, is widely available, provides immediate results, is highly accurate, and is repeatable at the bedside in clinical settings (115). Our values obtained are slightly higher than those described in a mouse protocol we used for comparison. Unfortunately, in this protocol, not many animal characteristics are detailed for discussion, not even age, sex or weight (116).

Diaphragmatic echography has been validated for detecting changes in diaphragmatic function, showing excellent correlation with *ex vivo* force measurement over a wide range of diaphragm excursion values ranging from wild type mice to neurologically diseased mouse models. In this study, diaphragm amplitude decreased with age and values were much lower than for wild-type mice. Diaphragm amplitude strongly correlated with *ex vivo* specific force values and validated diaphragm ultrasonography as a reliable technique for assessing time-dependent changes in a neurological mouse model of muscular dystrophy diaphragm function *in vivo*. This technique will be valuable for testing potential therapies for this condition (115,117).

When we normalize data of diaphragmatic echography as the change in diaphragmatic excursion from baseline to day 30 after starting isoproterenol infusion, results show a significant increase in muscle excursion during spontaneous breathing correlating with invasive measures. In contrast, diaphragmatic excursion at baseline was 2.12 ± 0.10 mm, with no significant differences between groups ($p = 0.600$) and the increase in diaphragm excursion was significantly higher in HF mice as compared with healthy animals at the end of the experiment.

Few studies measuring diaphragmatic excursion values have been published. In addition to previously mentioned studies in dystrophic-neurological mice, recent publications

have described more detailed values in patients, in dog experiments and in fetuses (118–120). Diaphragmatic echography is strongly positioned daily as a useful tool for diagnosis and monitoring cardiorespiratory status in both basic-translational and clinical research.

5.3 Invasive force measurements

Measurement of muscle function in respiratory muscle dysfunction has been documented through respiratory muscle tests that play crucial roles in cardiorespiratory fitness in which the diaphragm is taken as the primary inspiratory muscle and in which investigators assume that abnormal outcomes of these muscle tests reflect, directly or indirectly, diaphragm muscle dysfunction (71).

By contrast, direct and current assessments of the diaphragm using *ex-vivo* methodology allow the more extensive evaluation of contractility of isolated skeletal muscles with parameters such as muscle force, muscle power, contractile kinetics and fatigability. This method removes the nerve and blood supply and variables usually included indirectly in clinical test measures of diaphragm function as an 'inspiratory system' (phrenic motor neurons, neuromuscular junction, and muscles) and focuses on the isolated skeletal muscle itself (121).

Ex vivo force measurements were carried on diaphragm strips following conventional procedures, achieving values of peak specific twitch and tetanic forces in control mice (≈40 and ≈135 mN/mm2, respectively) which were very close to the ones reported for wild type mice when using a similar methodology, also maintaining diaphragm muscle in metabolic, environmental and physiological conditions previous to function test (≈35 and ≈170 mN/mm2, respectively). Interestingly, this *ex vivo* experimental setting allowed us to focus on muscle tissue contractibility, thus avoiding the potential effect of isoproterenol on neural activation.

5.4 Diaphragm dysfunction in other HF models

Enhancement in diaphragm function in the isoproterenol model strongly contrasts with clinical studies and findings in other rodent models of HF at similar time points. For instance, diaphragm dysfunction has been reported in rat models where HF is induced by different procedures such as left coronary artery ligation, aorto-caval fistula, aortic banding or monocrotaline administration. Regarding mouse models, diaphragm weakening has also been reported when HF is induced by left coronary artery ligation, transverse aortic constriction, or transgenic mice (50–56,58,76,122–126). In all these

studies focused on diaphragm muscle, most of them through isolated muscle test methodology, results suggest the ability to mimic muscular dysfunction observed in patients.

Therefore, the novel data reported from the present Thesis on the increased diaphragm contractibility observed in isoproterenol-induced HF at 30 days indicates that, as far as this respiratory muscle function is concerned, the model does not behave as in HF patients and other rodent models of the disease. Probably the reason is systemic administration of isoproterenol.

5.5 Isoproterenol-induced administration consequences

Isoproterenol is a non-selective beta-adrenergic agonist inducing early cardiac hypertrophy and hypercontractility followed by HF with cardiac dilation and ventricular dysfunction, secondary to chronic adrenergic overstimulation. The major drawback of isoproterenol therapy is its ability to generate free radicals, which cause oxidative stress leading to progressive mitochondrial damage and alterations in cardiac biochemical parameters that result in cardiac injury (79,127).

The diaphragm contractility enhancement we report here for the first time in the conventional isoproterenol-induced HF model was hypothesized based on previous indirect data on the effects of this non-selective beta-adrenergic agonist in non-cardiac muscles. Indeed, it was reported that the consequences of systemic or global administration of isoproterenol could affect skeletal muscles by activating their beta receptors responsible for fiber contraction physiology.

Chronic stimulation of beta receptors appears to stimulate the skeletal muscle in the direction of anabolism, growth, strengthening and hypertrophy. Resulting in increasing contractility and force production. Studies have shown that this stimulation/administration can prevent muscle atrophy in peripheral skeletal muscle in denervated rats. For instance, dietary administration of beta adrenoceptor agonist produces growth-promoting protein anabolic effects in muscle tissue and seems to cause hypertrophy of skeletal muscle in mice and diaphragm in hamsters. Moreover, when investigated in different experimental settings other than the HF model, isoproterenol increased canine and rodent diaphragm contractibility.

Interestingly, isoproterenol may also increase the contractile force of skeletal muscles in isolated settings, as it has been reported when added to the bath of *ex vivo* preparations

of limb mouse muscles (75,80,81,128–131). However, whether a continuous chronic infusion of isoproterenol as in the HF mouse model induces enhancement of other skeletal muscle's contractibility is unknown.

Although our main hypothesis of increased contractility of the diaphragm is given by the substance administered to produce and mimic HF, time could be a crucial variable in the adaptation and response of the diaphragm muscle. Future research on the model should include time-dependent or time-course analysis to see the evolution of diaphragmatic activity over time and rule out whether it is an adaptation or acute response to the pathophysiology stage of HF and/or isoproterenol as a beta receptor stimulator.

5.6 Microstructural changes to muscular (dys)function

The different physiological or physiopathological functional needs are related to strength power, speed of contraction and metabolism of muscles induce changes in the types of muscle fibers. In addition to enhanced force, diaphragmatic function analyses of isoproterenol-induced HF failure mice showed a fast decrease rate and hence a tendency to fatigue (Fig 5, Article 1). Interestingly, these performance in force production and fatigue resistance are both characteristic features of muscles with a greater distribution of type IIb fibers (132–134). The significant increase in *Myh4* (related to MyHC-IIb, the most fast-glycolytic muscle fiber type) in our isoproterenol-induced HF mice (Fig 6, Article 1) could partially explain the results observed in the *ex vivo* muscle testing. This fiber distribution contrasts with the results reported from diaphragm biopsies in patients with severe HF, showing a shift from fast to slow fibers with higher levels of type I and lower levels of type II fibers compared with healthy controls (135).

Likewise, some studies in animal models of chronic HF that adequately reproduce diaphragmatic weakness also described the same tendency with an increase in type I and IIa muscle fibers accompanied by decreases in type IId/x and IIb fibers (124,136,137). This shift from a more fast-glycolytic to a slow-oxidative metabolism in HF partially explains diaphragm weakness and his relationship with breathlessness/dyspnea and exercise intolerance. Therefore, although more detailed fiber analysis could be carried out, our results from myosin gene expression in diaphragms from isoproterenol-induced HF (Fig 6, Article 1) suggest that changes in fiber types differ from those described in HF patient and explain the more significant force production with vulnerability to fatigue resistance. These conditions sustained over time by patients reproduce adaptations to

alleviate the impact on symptoms and signs that usually affect patients attending rehabilitation programs use, participation and adherence.

5.7 Exercised-based cardiac rehabilitation participation

Only 50.5% of whole cohort patients included completed the exercise component of EB-CRP, despite the well described (and demonstrated in our study) better prognosis of those patients who attend and complete EB-CRP. Underuse of health strategies of secondary prevention as EB-CRP was described in other countries and continues to be a challenge. A very similar rate was obtained by Dunlay et al. (2014). In this study, 52.5% of participation was obtained in a population-based surveillance study of patients after their first ever myocardial infarction a few years ago in Minnesota, USA (138). At the same time, previous studies have shown an even-lower participation rate of <30%. Ritchey et al. (2020), and colleagues in an observational-retrospective methodology analyzing 366.103 eligible beneficiaries for EB-CRP, found that only 24.4% participated. In this study, the authors conclude that only 1 in 4 CR-eligible medicare beneficiaries participated in CR, observed and detailed marked disparities. Reinforcement of current effective strategies will be critical to addressing these disparities and achieving the 70% of participation goal (138–141).

Patients who completed the ET were younger and with fewer comorbidities, observations that are consistent with those of other studies (101,138). This finding might seem counterintuitive because higher-risk patients (i.e., those with comorbidities) should be more prone to accept ET to decrease their risk (142). A-NT and R-NT groups were older and had more comorbidities, some of which might have hindered their ability to exercise (e.g., peripheral vascular disease and anemia).

5.8 Reasons of not performing exercise training

Reasons for not accepting to undergo ET or for accepting but not completing it were quite similar in the R-NT and A-NT groups. The main reason in both groups (41% of patients) was the unwillingness of the patients to participate, with no explanations for this. This finding is consistent with other studies where 47.5% of patients did not participate in ET (103,138,143).

A possible explanation for the lack of information and details could be related to the subjectivity and perception of patients after suffering from heart disease. Through qualitative scientific methods, some recent studies have been able to deepen into the key

elements, historically inattentive. These methods have made it possible to systematize variables without reducing them to a cohort score or a simple quantitative description. For instance, these studies have strongly contributed to understanding kinesiophofia and dyspnea, fundamental elements to consider adherence to exercise programs that are not frequently quantified or measured (144,145). Noteworthy, active components of EB-CRP have been identified and the perceptions of different training modalities and settings for ET have been explored recently using qualitative and mix-methods approaches (146,147). These contributions not only help to resolve structural barriers and take into account apparently invisible elements but also bring us closer to practicing patient-centered care (PCC) in a better way.

In the gender-sex analyses, it is essential to focus on women not only in a reductionist way to meet the equity quota but to balance the barriers of historical structural inequities and socio-structural disadvantages that impact personal recovery in cardiac rehabilitation (148). Deepening and exploring, for example, patients who reported having to do household work as an impossible reason for attending EB-CRP could also improve structural inequities, adherence and participation.

Our data included 18.71% of females compared with other studies that reported an 11–20% lower enrollment in which women were more likely to withdraw from a program than men (35% vs. 29%) (89,149). For instance, gender, age, comorbidities, disease perception, social class, education level, accessibility and proximity, among other reasons, play a crucial role in adherence to ET. For women, barriers to CR participation are multiple and complex, explaining partially their low participation rates (150,151).

5.9 Patient centered-care and EB-CRP

Few patients (<30%) were excluded from ET due to medical reasons, such as the presence of comorbidities. However, the percentage remained significant and highlight the need to focus on what can be changed in the system to guarantee that structural barriers to ET disappear so that genuine PCC can be carried out, which would significantly increase the number of patients who complete ET and also bridge the gender gap in participation. PCC can help identify intangible barriers, such as those reported by Resurrección et al. (2017), in which traditional ways of CRP with a predominantly male presence could make women feel uncomfortable (152). Finally, our results showed an excellent long-term prognosis in patients who completed ET, with a composite of all-cause mortality, hospitalization due to ACS, or need for new

revascularization in only 3.6%, after more than two years of follow-up mortality of 0.8%. In contrast, patients in the R-NT and A-NT groups showed at least four times more risk with the composite endpoint and all-cause mortality, with no significant difference between the two groups. These results are consistent with other studies that found that the risk of death or having a recurrent CV event in patients who do not complete ET is over double the risk in patients who complete it (101).

5.10 Risk stratification, exercise training and cardiac rehospitalization

In the risk stratification study, 56.7% of patients admitted due to an ACS were low-risk according to an easy-to-calculate score that included cardiorespiratory capacity and left ventricular ejection fraction. Cardiac rehospitalization in the no-low risk group was significantly higher than in the low-risk group (HR 3.83 (95 CI 1.51–9.68)). Completing the exercise program was also independently associated with a better prognosis, and 50% of the patients in the no-low risk group who completed it became low-risk. This is consistent with other studies and meta-analyses in the literature that have shown the positive effect of exercise participation in reduced cardiac rehospitalization and all-cause hospital readmission, as our data suggest (153,154). Baseline characteristics did not differentiate patients in the low- and no-low risk groups and the only differences were age and diabetes, but these variables were not strikingly different. In some of the most used risk scores, this different amount detailed in the third article of this Thesis would not have made significant change in the stratification.

As expected, due to the variables included in the stratification protocol, LVEF and cardiorespiratory capacity differed between both groups. However, it is worth noting that the median LVEF was >50% in both groups. Interestingly, although patients in the low-risk group had higher METs (10.3 vs. 8.3, $p < 0.001$), both groups had good cardiorespiratory capacity. Several studies have shown that METs vary with age, but patients in their 50s and 60s have a cardiorespiratory capacity of 6 to 10 METs (155,156).

After multivariable analysis, patients in the no-low risk group had a worse prognosis with an HR 3.83 (95% CI 1.51–9.68) for the primary endpoint. Other risk scores have shown that prognosis after an ACS worsens with increased risk. Risk stratification of the GRACE score indicated that the mortality risk of the intermediate-risk and high-risk groups was higher, with an HR 3.23 (1.59–6.55) for the intermediate-risk group and HR 15.31 (4.43–51.62) for the highest risk group. Similar results were observed with MACCE

risk score (157). Still, different endpoints, follow-up periods, and baseline characteristics can explain the differences in outcomes with our results, especially in the high-risk group.

5.11 Risk stratification variation with exercise compliance

Finally, this study showed that all patients initially classified in the low-risk group remained in this group. However, completion of the exercise training was associated with reclassification from the no-low to low-risk group more frequently than in the control group. The no-low risk group who completed the exercise training had the most significant improvement with a relative increase of 14.3% in the METS achieved in the treadmill stress test. This reclassification was associated with a better outcome. Although several studies and meta-analyses have shown that exercise training is associated with a better prognosis, few studies have analyzed whether the change in risk categories is associated with prognosis (158). The proposed risk score could identify no-low risk patients soon after an ACS and add further prognostic information after an exercise training program.

5.12 Animal model limitations and future perspectives

Remarkably, beyond the limitations summarized in the first article of this Thesis, experimental research practice could be the main limitation in the underrepresentation of female subjects in animal research. This sex difference has gained attention in recent years, strongly supported by genetic or physiological features such as female estrous cyclicity. Even though empirical research across multiple rodent species and traits demonstrates that females are not more variable than males and that for most traits, female estrous cyclicity need not be considered (159–161). Including both sexes in animal research studies should drive important discoveries in both basic-translational and clinical research.

In addition, incorporating both sexes, including measurement of other skeletal muscles different from respiratory muscles and evaluating the impact on various exposure times to isoproterenol, could help to understand the role of this drug in muscle tissue and define its translational interest in HF research, mainly when aimed at integrating cardiorespiratory alterations.

5.13 Clinical limitations and future perspectives

The main limitation of the clinical observational component of this Thesis is that results might not apply to other settings as a single-center study. Still, given that all patients

followed the same protocol and that the information was documented in the medical record in a structured way, we believe that the risk of bias in this study is negligible, even when some patients were included retrospectively.

Future studies might include a more significant number of women in the sample size for addressing statistically gender-sex differences. Mixed methods, including qualitative studies, that include participant's perception and subjectivity in EB-CRP, can be crucial for improving adherence, attendance and prognosis in secondary prevention.

Chapter IX:
Conclusions

Muscular function evaluation in CVD animal model assess: Diaphragm structure and function compared with healthy controls (*Aim i and ii*)

[1] A system for the mechanical assessment of respiratory muscle function was developed and implemented.

[2] Diaphragm function in the conventional isoproterenol-induced heart failure model was considerably enhanced in both *ex-vivo* and *in-vivo* measurements.

[3] The diaphragmatic excursion was higher in the conventional isoproterenol-induced heart failure model than in healthy animals in *in-vivo* echography measurements.

[4] Diaphragm force production in the conventional isoproterenol-induced heart failure model was considerably higher than in healthy animals in *ex-vivo* force measurements.

[5] Gene expression of Myh4 relative to structural fibers MyHC-IIb showed a significant 2-fold increase in conventional isoproterenol-induced heart failure model compared with healthy animals.

[6] The model elicits a considerable increase in diaphragm contractility, thereby questioning its translational interest in HF research, especially when aimed at integrating cardiorespiratory muscle alterations at 30 days.

Impact of EB-CRP in ACS patients *(Aim iii and iv)*

[7] Completion of ET after ACS is associated with an excellent and improved prognosis at a median follow-up of 31 months.

[8] Patients who outrightly rejected participating in ET and those who began but did not complete it had similar baseline characteristics and prognosis, which was worse than those completing ET.

[9] The main reasons for not participating in ET were unknown refusal-reason and work and schedule/distance incompatibility. These results highlight the need to focus on the needs of the patients in order to minimize structural barriers to ET.

To test an easy-to-calculate risk score in EB-CRP *(Aim iii)*

[10] The new routine risk stratification method offers prognostic information, could identify patients suitable for exercise training in an unsupervised setting and identify low-risk patients with excellent prognosis at follow-up.

[11] Given that this easy-to-calculate routine risk stratification method offers prognostic information, suggesting that it should be used in all patients after an ACS.

[12] Exercise-based component of CRP showed the ability to change risk stratification, improving functional classification and prognosis of these patients who initially belonged to the no-low risk and ended as low risk.

Chapter X:
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Chapter XI:
Appendices

APPENDIX A

6 Acquisition system software

Examining *ex-vivo* muscle contraction and force production is widely used to evaluate muscle function. This approach is commonly applied as a standard method for evaluating isolated muscle in a bath. The use of murine models for mimicking human diseases has increased our interest in obtaining accurate measurements of muscular performance. Here, we briefly illustrate a custom-built system to acquire the signal of force transducer signal when we applied an electrical-stimulus that mimicking physiological stimulation using LabView and an offline analysis based on MatLab. This system is functional for contractile tissue evaluation in *ex vivo* experimental settings. This tool, which was developed in close collaboration with the electronic engineer in the lab, Mr. Miguel A. Rodríguez Lázaro, achieves high sensitivity and reproducibility and is customizable and user-friendly.

6.1 Generating and acquisition of biological signals

LabView is a platform used to acquire, visualize and handle data. Two modules form the acquisition block diagram. The first aim was to produce controlled electrical stimuli by varying all parameters of width, cycle, frequency and duration of the pulse (Figure 10).

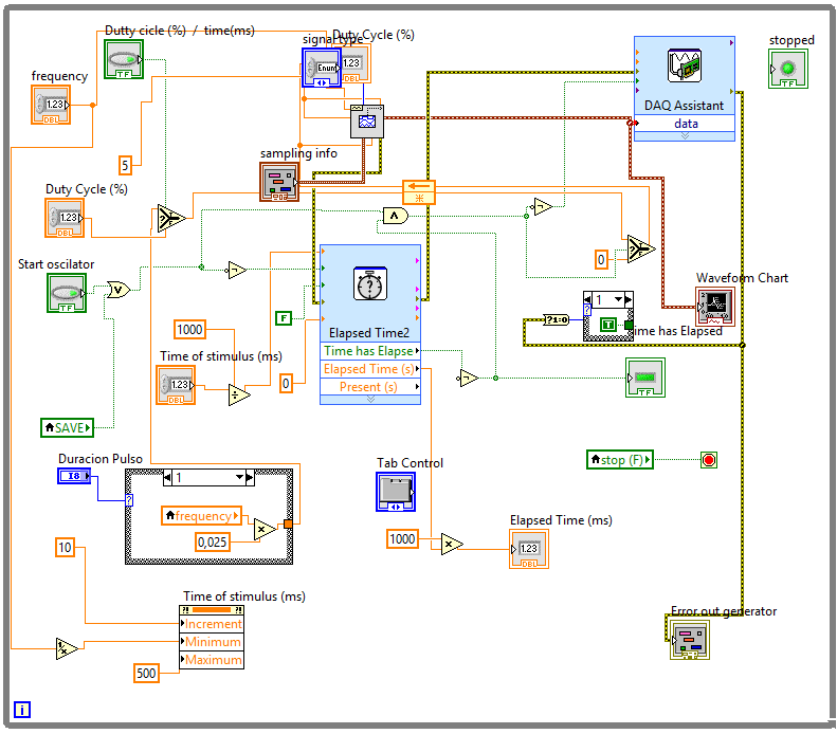

Figure 10. Software program block diagram for generating electrical stimuli.

The second block permits the acquisition of the force signals at the sample of 10.000 Hz, cleaning and rectifying the signal obtained as the response of muscle function (Figure 11). This data can be visualized live and users can set different acquisition-time (Figure 12).

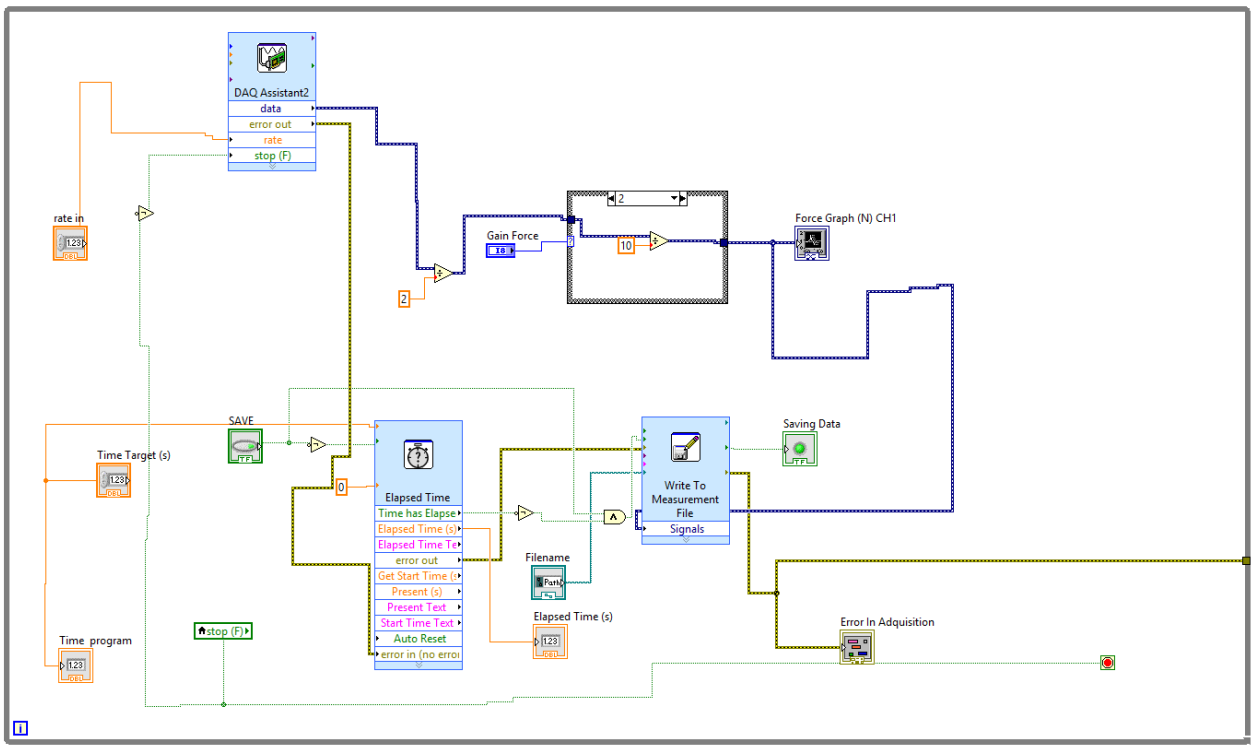

Figure 11. Software program block diagram for recording muscle response.

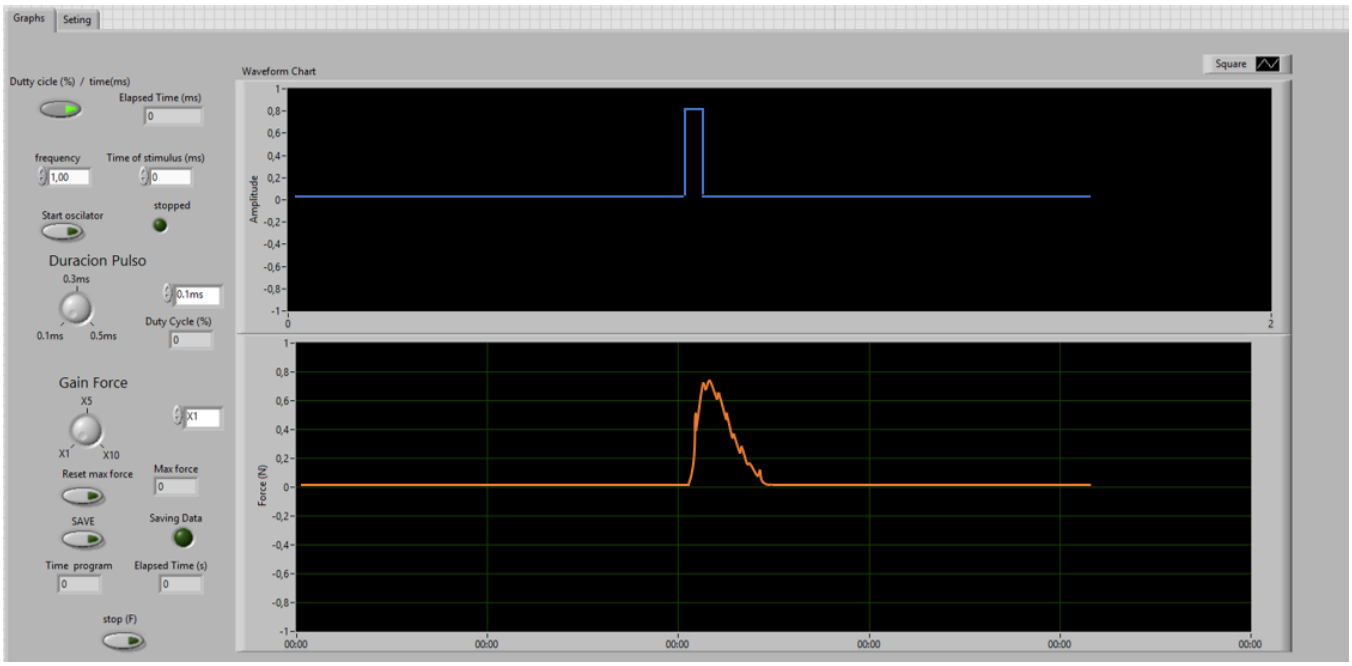

Figure 12. Software user interface.

APPENDIX B

7 Contribution to other research projects, publications and conference communications

Product of the work of this Thesis and results from other contributions in collaborative research projects:

7.1 Publications

[1] **I. Cabrera-Aguilera**, B. Benito, M. Tajes, R. Farré, D. Gozal, I. Almendros and N. Farré. "Chronic Sleep Fragmentation Mimicking Sleep Apnea Does not Worsen Left-Ventricular, Function in Healthy and Heart Failure Mice". Frontiers Neurology., Jan. 2020. IF = 4.003, Q2.

[2] O. Khannous-Lleiffe, J. Willis, E. Saus, **I. Cabrera-Aguilera**, I. Almendros, R Farré, D. Gozal, N. Farré and T. Gabaldón. "A Muse Model Suggest the Heart Failure and its Common, Comorbidity Sleep Fragmentation Have No Synergistic impacts on the Gut Microbiome". Microorganism., Feb. 2021. IF = 4.128, Q2.

[3] B. Falcones, H. Sanz-Fraile, E. Marhuenda, I. Mendizábal, N, I. Mendizával, **I. Cabrera-Aguilera**, N. Malandain, J. Uriarte, I. Almendros, D. Navajas, D. Weiss, R. Farré and J. Otero. "Bioprintable Lung Extracellular Matrix Hydrogels Scaffolds for 3D Culture of Mesenchymal Stromal Cells". Polymers., Jul. 2021. IF = 4.329, Q1.

7.2 Congress communications

[4] **I. Cabrera-Aguilera**, B. Benito, M. Tajes, R. Farré, D. Gozal, N. Farré and I. Almendros. "Sleep Fragmentation Does not modify Cardiac Function in a mouse model of Heart Failure". World Sleep Congress 2019.

[5] **I. Cabrera-Aguilera**, B. Benito, M. Tajes, R. Farré, D. Gozal, I. Almendros and N. Farré I. "Sleep Fragmentation mimicking Sleep Apnea does not alter Cardiac Function in either control or Heart Failure mice". European Respiratory Society Congress 2019.

[6] **I. Cabrera-Aguilera**, B. Falcones, R. Farré, I. Almendros and N. Farré. "Diaphragm dysfunction in isoproterenol-induced Heart Failure". European Respiratory Society Congress 2020.

[7] B. Falcones, H. Sanz, **I. Cabrera-Aguilera**, I. Almendros, D. Navajas and R. Farré. "3D culturing mesenchymal stem cells in lung extracellular matrix hydrogels affects their homing potential". European Respiratory Society Congress 2020.

[8] I. Almendros, B. Falcones, H. Sanz, E. Marhuenda, I. Mendizával, **I. Cabrera-Aguilera**, D. Navajas. R. Farré and J. Otero. "Preconditioning mesenchymal stem cell by bioprinting and 3D culturing into lung extracellular matrix hydrogels improves their adhesion and homing capacity". American Thoracic Society Congress 2020.

[9] **I. Cabrera-Aguilera**, C. Ivern, N. Badosa. E. Marco, L. Salas-Medina. D. Mojón, M. Vicente, M. Llagostera, N. Farré and S. Ruiz. "Impacte i raons de no realitzar l'entrenament físic després d'una síndrome coronària aguda en context d'un programa de rehabilitació cardíaca interdisciplinari. Resultats del registre Risk-Op-ACS". Societat Catalana de Cardiología Congress 2021.

[10] E. Marco, **I. Cabrera-Aguilera**, C. Ivern, N. Badosa, N. Farré and S. Ruiz. "Utilidad Pronóstica de un nuevo sistema de estratificación del riesgo en pacientes con cardiopatía isquémica remitidos a Rehabilitación Cardiaca". 2º Congreso Iberoamericano de Rehabilitación y 60º Congreso Nacional de la Sociedad Española de Rehabilitación y Medicina Física 2022.