Department of Internal Medicine/ Cardiology and Angiology
Faculty of Medicine
Otto-von-Guericke-University
Magdeburg, Germany

Comorbidities of heart failure with the focus on skeletal muscle structure and function: From gene expression to clinical manifestation

The cumulative Habilitation Thesis
As part of the academic fulfilment

for
the academic degree Dr. med. Habil.
(doctor medicinae habilitatus)

At the faculty of Medicine
of the Otto-von-Guericke-University
Magdeburg, Germany

by

Dr. med. Tarek Bekfani
From Swaida/Syria

Magdeburg, September 2022

To the source of my inspirations and perseverance,
to Rieke, my parents, my brothers, Joud and Joulie.

To all my teachers, mentors, students, colleagues, and friends
who always enlightened my way, pushed me in the right direction and
gave all the support that I needed

References included in this Habilitation thesis:

1. **Bekfani T**, Bekhite Elsaied M, Derlien S, Nisser J, Westermann M, Nietzsche S, Hamadanchi A, Fröb E, Westphal J, Haase D, Kretzschmar T, Schlattmann P, Smolenski UC, Lichtenauer M, Wernly B, Jirak P, Lehmann G, Möbius-Winkler S, Schulze PC. Skeletal Muscle Function, Structure, and Metabolism in Patients With Heart Failure With Reduced Ejection Fraction and Heart Failure With Preserved Ejection Fraction. Circ Heart Fail. 2020 Dec;13(12):e007198.

2. von Haehling S, Garfias Macedo T, Valentova M, Anker MS, Ebner N, **Bekfani T**, Haarmann H, Schefold JC, Lainscak M, Cleland JGF, Doehner W, Hasenfuss G, Anker SD. Muscle wasting as an independent predictor of survival in patients with chronic heart failure. J Cachexia Sarcopenia Muscle. 2020 Oct;11(5):1242-1249.

3. **Bekfani T**, Bekhite M, Neugebauer S, Derlien S, Hamadanchi A, Nisser J, Hilse MS, Haase D, Kretzschmar T, Wu MF, Lichtenauer M, Kiehntopf M, von Haehling S, Schlattmann P, Lehmann G, Franz M, Möbius-Winkler S, Schulze C. Metabolomic Profiling in Patients with Heart Failure and Exercise Intolerance: Kynurenine as a Potential Biomarker. Cells. 2022 May 18;11(10):1674.

4. **Bekfani T,** Hamadanchi A, Ijuin S, Bekhite M, Nisser J, Derlien S, Westphal J, Bogoviku J, Morris DA, Fudim M, Braun-Dullaeus RC, Möbius-Winkler S, Schulze PC. Relation of left atrial function with exercise capacity and muscle endurance in patients with heart failure. ESC Heart Fail. 2021 Dec;8(6):4528-4538.

5. Valentova M, von Haehling S, Bauditz J, Doehner W, Ebner N, **Bekfani T**, Elsner S, Sliziuk V, Scherbakov N, Murín J, Anker SD, Sandek A. Intestinal congestion and right ventricular dysfunction: a link with appetite loss, inflammation, and cachexia in chronic heart failure. Eur Heart J. 2016 Jun 1;37(21):1684-91.

6. Saitoh M, Dos Santos MR, Emami A, Ishida J, Ebner N, Valentova M, **Bekfani T,** Sandek A, Lainscak M, Doehner W, Anker SD, von Haehling S. Anorexia, functional capacity, and clinical outcome in patients with chronic heart failure: results from the Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF). ESC Heart Fail. 2017 Nov;4(4):448-457.

7. **Bekfani T**, Pellicori P, Morris D, Ebner N, Valentova M, Sandek A, Doehner W, Cleland JG, Lainscak M, Schulze PC, Anker SD, von Haehling S. Iron deficiency in patients with heart failure with preserved ejection fraction and its association with reduced exercise capacity, muscle strength and quality of life. Clin Res Cardiol. 2019 Feb;108(2):203-211.

8. **Bekfani T**, Nisser J, Derlien S, Hamadanchi A, Fröb E, Dannberg G, Lichtenauer M, Smolenski UC, Lehmann G, Möbius-Winkler S, Schulze PC. Psychosocial factors, mental health, and coordination capacity in patients with heart failure with preserved ejection fraction compared with heart failure with reduced ejection fraction. ESC Heart Fail. 2021 Aug;8(4):3268-3278.

9. Peters AE, Pandey A, Ayers C, Wegermann K, McGarrah RW, Grodin JL, Abdelmalek MF, **Bekfani T**, Blumer V, Diehl AM, Moylan CA, Fudim M. Association of liver fibrosis risk scores with clinical outcomes in patients with heart failure with preserved ejection fraction: findings from TOPCAT. ESC Heart Fail. 2021 Apr;8(2):842-848.

10. **Bekfani T**, Schöbel C, Pietrock C, Valentova M, Ebner N, Döhner W, Schulze PC, Anker SD, von Haehling S. Heart failure and sleep-disordered breathing: susceptibility to reduced muscle strength and preclinical congestion (SICA-HF cohort). ESC Heart Fail. 2020 Oct;7(5):2063-2070.

Table of Contents

ABBREVIATIONS

ACADM: medium-chain acyl-CoA dehydrogenase

ACEI: angiotensin converting enzyme inhibitors

AHF: acute heart failure

AHI: apnea/hypopnea index

Akt: protein kinase B

AMI: acute myocardial infarct

ARB: angiotensin receptor blocker

ASM: appendicular skeletal muscle mass

BIA: bioelectrical impedance analysis

BNP: brain natriuretic peptide

BWT: bowel wall thickness

CAF: C-terminal agrin-fragment

CD36: fatty acid translocase

COPD chronic obstructive pulmonary disease

CPET: cardiopulmonary exercise testing

CPT1B: carnitine palmitoyl transferase IB

CSA: central sleep apnea

CTSL: cathepsin L

DCM: dilated cardiomyopathy

DEXA: dual energy X-ray absorptiometry

DM: diabetes mellitus

ECW: extra-cellular water

FBXO-32: F-box only protein 32 (Atrogin1)

FIB-4: fibrosis-4 scores

FOXO: forkhead box protein O

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

GDF-15: growth and differentiation factor 15

GFR: glomerular filtrating rate

GLUT1,4: glucose-transporters 1,4

HADS: hospital anxiety and depression scale

Hb: hemoglobin

HC: healthy controls

HF: heart failure

HFmrEF: HF with mid-range reduced LVEF

HFpEF: HF with preserved LVEF

HFrEF: HF with reduced LVEF

HTX: heart transplantation

ICM: ischemic cardiomyopathy

ICW: intracellular water

ID: iron deficiency

IGF-1: insulin-like growth factor 1

LV: left ventricular

LAVI: left atrial volume index

LVAD: left ventricular assist device

LVEDVI: left ventricle end-diastolic volume index

LVEF: left ventricle ejection fraction

LVMI: left ventricle mass index

MCS: mental component score

MFN2: mitofusin 2

6-MWT: 6-minute walk testing

MYH 7, 2: myosin fibers type I and type II

NAFLD: nonalcoholic fatty liver disease

NASH: nonalcoholic steatohepatitis

NFS: non-alcoholic fatty liver disease fibrosis score

NYHA: New York heart association classification

OR: odds ratio

OSA: obstructive sleep apnea

PCS: physical component score

PDK4: pyruvate dehydrogenase kinase 4

PPAR α: peroxisome proliferator-activated receptor α

PVO2: maximal Oxygen uptake

QoL: quality of life

RAP: right atrial pressure

RME: reduced muscle endurance

ROC: receiver operating characteristics

RV: right ventricular

SDB: sleep-disordered breathing

SF-36: short form of health survey

SICA-HF: the studies investigating comorbidities aggravating HF

SPPB: short physical performance battery

TGF-ß: transforming growth factor ß

TRIM63: Tripartite Motif Containing 63

TSAT: transferrin saturation

UBB: ubiquitin B

UCP3: uncoupling protein

VE/VCO2: ventilatory efficiency slope

1. INTRODUCTION

1.1. Definition and classification of heart failure

Heart failure (HF) is a clinical syndrome where patients present mainly with dyspnea, reduced exercise capacity, and fatigue. Clinically observed signs are ankle edema, elevated jugular venous pressure, crackles in the lungs, or a third heart sound (S3) during auscultation. Regardless of the etiology, patients with HF show reduced cardiac output [1]. The manifestations and the severity of HF are usually aggravated by existing comorbidities. HF-comorbidities were studied extensively in several studies such as the studies investigating comorbidities aggravating HF (SICA-HF). [2] A considerable proportion of the current cumulative habilitation was performed as part of SICA-HF.

There are different ways of classifying HF. These are some examples: 1- according to the left ventricle ejection fraction (LVEF), or 2- according to the time course of HF.

1.1.1 Classification of HF according to LVEF

In summary, the guidelines of the European Society of Cardiology – HF 2021 (ESC-HF) classified patients with HF into 3 groups according to LVEF as follows: HF with reduced LVEF ≤40% (HFrEF), HF with mildly reduced LVEF: 41-49% (HFmrEF), and HF with preserved ejection fraction (HFpEF) where LVEF ≥50% (Table 1) [1].

1.1.2 Classification of HF according to the time course of HF

Chronic HF describes patients who have established diagnosis of HF or those who have gradual onset of symptoms. If HF-therapy is stable over the last 4 weeks and the patient does not suffer from worsening signs and symptoms, this case will be described as stable chronic HF. Any deterioration from the above condition is defined as decompensated HF or acute heart failure (AHF) or worsening HF [1].

Table 1: Classification of HF according to the ESC-HF- guidelines 2021:

Type of HF/Criteria	HFrEF	HFmrEF	HFpEF
	Symptoms ± signs	Symptoms ± signs	Symptoms ± signs
	LVEF ≤ 40 %	LVEF 41-49 %	LVEF ≥ 50%
		- Elevated levels of BNP/ NT-proBNP* - At least one additional criterion: - Relevant structural heart disease¶ - Diastolic dysfunction§	- Elevated levels of BNP/ NT-proBNP* - At least one additional criterion: - Relevant structural heart disease¶ - Diastolic dysfunction

∗ BNP/NT-proBNP in clinically stable outpatient with HF should be at least 35 and 125 pg/ml, respectively.
¶ structural heart disease: either left ventricular hypertrophy (interventricular septum thickness of posterior wall ≥13 mm) or left atrial enlargement (left atrial volume index -LAVI > 34 ml/m^2).
§ To evaluate the diastolic dysfunction in the echocardiography, one needs to measure the mitral inflow wave (E-wave) and tissue doppler waves by acquiring medial and lateral mitral annulus velocities (e´ wave). An index of E/é > 13 shows key functional alterations. This is described in detail in the ESC-HF guidelines 2021 and echocardiography guidelines [3, 4].

1.2 Incidence and prevalence of HF

HF is a major health, social and economic problem with about 26 million people worldwide currently suffering from HF [5, 6]. Epidemiological analysis shows an incidence of HF up to 4/1000 person-years [7]. Given an aging population and the rising burden of comorbidities, incidence and prevalence are increasing [8, 9]. A further explanation for the increased prevalence is the improved survival after HF has been diagnosed, which is in part due to the continuous improvement and new discoveries of medical and device therapy [10-15]. The prevalence of HF in developed countries is currently estimated to be about 10% and 30 % among people older than 70 and 80 years, respectively. It is expected that the prevalence of HF will increase about 46% from 2012 to 2030 [16, 17].

In spite of advancements in medical and device therapy over the last decades the rates of HF admissions and readmission because of AHF are still very high [18, 19]. The readmission rates one month after discharge, as an indicator of the efficacy of in-hospital and discharge management of patients with AHF, are still with about 25%, very high. About 50% of these patients will be re-hospitalized within the first year after discharge [20-22]. Many of the hospitalizations are due to non-cardiovascular causes [23].

The resulting economic burden on health system and care delivery is immense. The costs related to HF in the US are expected to increase by 130% and reach in year 2030 about 70 billion US [24, 25].

Mortality due to HF has declined in the last couple of decades reflecting the improvement in medical and device therapy [26]. However, HF is still accompanied with poor outcome. The estimated survival beginning from the diagnosis of HF is about 75% and 35-50 % in the 1and 5 years, respectively [26]. The causes of mortality in these patients are often due to non-cardiovascular (comorbidities) causes, especially in patients with HFpEF [27].

1.3 Etiology of HF

Different predisposing factors for HF in the general population have been identified. These include arterial hypertension, diabetes mellitus (DM), hypercholesteremia, coronary artery diseases, obesity, smoking, and cardiac arrhythmias [28]. Patients with HFpEF have usually more comorbidities such as arterial hypertension, atrial fibrillation, and are often obese. Women tend to be more affected with HFpEF than men [29]. On the other hand, those patients with HFrEF have more often coronary artery disease leading to ischemic cardiomyopathy (ICM). Sometimes there is no clearly identifiable etiology and patients still suffer from reduced LVEF and show enlarged left ventricle diameters. This entity is called dilated cardiomyopathy (DCM). Other examples of cardiomyopathies are toxic cardiomyopathy (chemotherapy-induced, ethanol), infectious/inflammatory (influenza, giant cell myocarditis), infiltrative (amyloidosis), or familial (hypertrophic cardiomyopathy) [30].

1.4 Comorbidities of HF

HF-Comorbidities can be cardiac such as coronary artery disease, arterial hypertension, atrial fibrillation, valvular heart disease, or non-cardiovascular like DM, chronic obstructive pulmonary disease (COPD), renal insufficiency, anemia, iron deficiency, sleep-disordered breathing, skeletal muscle dysfunction (sarcopenia), or wasting disorders in general (cachexia), or psychosocial disorders (depression, anxiety) [4] **(Figure 1).**

In some epidemiological analyses patients with HF showed 5 or more comorbidities [31]. The risk of hospitalization is associated strongly with the number of comorbidities [32]. Non-cardiovascular comorbidities are responsible for more than half of the hospitalizations and often lead to worse outcomes in patients with HF especially in those with HFpEF [23]. Therefore, screening for comorbidities systematically in patients with HF is very important and could influence the outcome and the prognosis in patients with HF.

Comorbidities also interfere with the diagnosis of HF. COPD for example is a confounding factor for dyspnea [33]. HF and COPD have many further similarities, for example smoking is a risk factor for both diseases. Lung crackles as clinical findings might be seen in both diseases. In the advanced stages of both HF and COPD, patients may present with unintentional weight loss, malnutrition, and cachexia. Comorbidities can aggravate HF and worsen quality of life (QoL) [34]. This is true for almost all cardiac and non-cardiovascular comorbidities (atrial fibrillation, valvular heart diseases, kidney dysfunction, iron deficiency). Furthermore,

comorbidities may affect the therapy for HF. Prescribing angiotensin converting enzyme inhibitors or angiotensin receptor blockers is sometimes not possible in patients with severe renal insufficiency and requires special attention in patients with hyperkalemia [35].

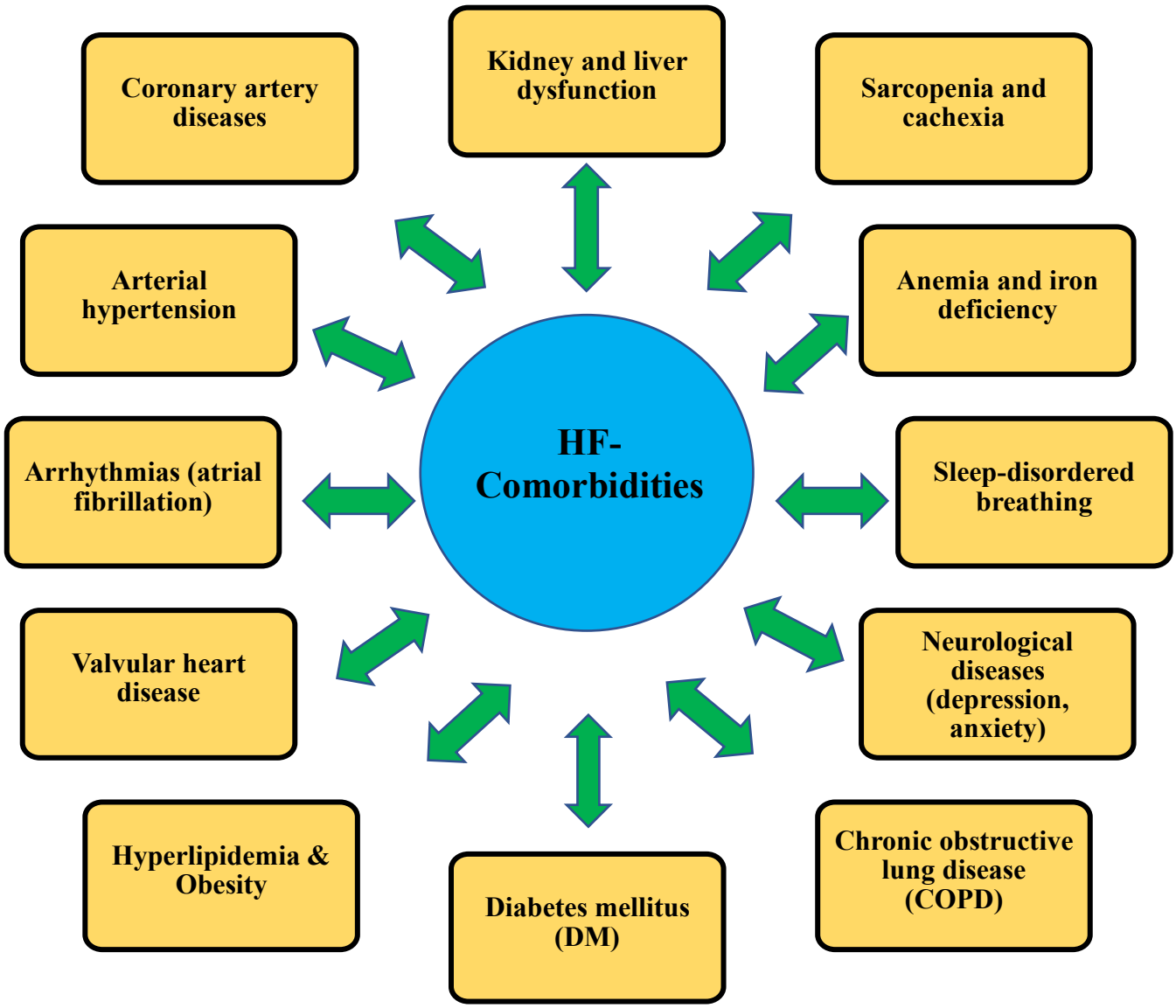


Figure1: A variety of comorbidities of HF. These can be divided into cardiac and non-cardiac.

The heart and kidney are in continuous interaction. In healthy individuals, the kidney is depending on the blood flow and perfusion pressure supplied by the heart, which in turn relies on a normal kidney function in regulating fluid and salt content of the body [36]. Fluid overload as a result to HF leads to harm and functional deterioration of both organs [36]. The maladaptive interactions between kidney and heart occurring in various diseases is called the cardiorenal syndrome. About 50% of patients with HF, both with HFpEF and HFrEF, have an estimated glomerular filtration rate of 60 (L/min/1.73 m^2), which is consistent with underlying renal dysfunction [37].

Moreover, the treatment of comorbidities may worsen HF, for example non-steroidal anti-inflammatory drugs (NSAIDS) as therapy for arthritis or chemotherapy for patients with cancer [38]. Additionally, the therapy of HF and the comorbidities can lead to reduced efficacy and further side effects (e.g., beta-blocker and beta-adrenergic agonists for the management of HF and COPD, respectively) [33]. On the other hand, treating comorbidities has been proven to improve symptoms of HF (e.g., iron deficiency) [39]

Here is a summary of the most common comorbidities of HF.

1.4.1 Diabetes mellitus

Diabetes mellitus (DM) is one of the most common co-morbidities of HF. According to different studies and surveys, the prevalence ranges from 30 to 50 % in both patients with HFpEF and HFrEF [40, 41].

Patients with DM and chronic HF especially those with ischemic cardiomyopathy (ICM) tend to have higher short-term rehospitalization and short-term mortality. However, long-term prognostic consequences are less clear in this group of patients [42-45].
Glycemic dysregulation is mainly responsible for pathophysiological mechanism of DM in patients with HF. This consists of mitochondrial dysfunction, (micro)vascular disease, and oxidative stress [46, 47]. HF predisposes to DM and vice versa [48].

1.4.2 Arterial hypertension

Arterial hypertension is probably the most common comorbidity in HF and the most modifiable risk factors in this group of patients [49]. HFpEF is the most common cardiac complication of arterial hypertension. Arterial hypertension is a risk factor for developing myocardial infarct and might lead to ischemic heart disease and finally to HFrEF.

1.4.3 Atrial fibrillation

Atrial fibrillation is as common as 1-2% in the general population. Its prevalence is expected to increase by 2.5% in the year 2050 [50, 51]. Both atrial fibrillation and HF have common risk factors such as age, obesity, arterial hypertension, DM, structural and valvular heart diseases. Prevalence of atrial fibrillation in patients with HF is about 30%. It is known in the meanwhile that the pathophysiological changes occurring in HF and atrial fibrillation will promote the development of the other disease [52, 53]. The coexistence of both diseases has a worse prognosis and leads to elevated rates of mortality and stroke [54].

1.4.4 Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) co-exists in up to 50% of patients with chronic HF [41]. The prevalence is about seven times higher compared to the general population [33]. As a result to a missing structural screening system, prevalence indices vary considerably [55]. In addition, spirometry is not always conclusive in patients with HF due to pulmonary congestion [56]. This makes the diagnosis of COPD in patients with HF even more difficult.

In summary, a systematic screening for and an effective therapy of comorbidities in patients with HF is central in the management of patients with HF. These patients require tailored therapies for both HF and for their comorbidities.

This habilitation thesis investigates and describes common but often forgotten comorbidities such as skeletal muscle changes, sarcopenia, cachexia, iron deficiency, sleep related breathing disorders, mental disorders such depression and anxiety, and liver changes. These comorbidities have been my research topic in almost the last 10 years.

2. SKELETAL MUSCLE WASTING (SARCOPENIA) AND CACHEXIA

Age-related loss of skeletal muscle mass and function is part of the normal aging process and has been termed sarcopenia or muscle wasting [57], and it affects about 10% of elderly healthy subjects aged 60–70 years [58]. The prevalence is much higher in chronic diseases such as HF [59].

Appendicular skeletal muscle mass (ASM) that includes non-fat and non-bone tissue in both arms and legs combined in grams was analyzed in patients using dual energy X-ray absorptiometry (DEXA) to evaluate skeletal muscle mass according to the definition of sarcopenia (muscle wasting) [60]. In accordance with previously published consensus statements and using reference values (7.26 kg/m^2 in men vs. 5.45 kg/m^2 in women) from the previously published younger (age range: 18–40 years) Rossetta cohort [61], sarcopenia was defined as muscle mass 2 standard deviations below the mean of the reference values in this reference population. The ratio resulting from indexing appendicular lean mass to body height (in meters squared) was used to separate patients with and without sarcopenia [62].

The 2021 HF guidelines of the European Society of Cardiology acknowledged sarcopenia as an important comorbidity of HF that requires particular attention [4], because wasting processes are accelerated and more pronounced in chronic diseases including HF [63]. The prevalence of sarcopenia in a mixed cohort of patients with symptomatic chronic HF was found to be 19.5% in a recently published study by our group [64].

In a previous study, Bekfani et al enrolled a total of 117 symptomatic outpatients with HFpEF prospectively in Germany, England, and Slovenia as part of a multi-center European study (SICA-HF). ASM was assessed by DEXA. Echocardiography, 6-minute walk testing (6-MWT), muscle strength assessment, cardiopulmonary exercise testing (CPET) and QoL evaluation were performed. Muscle wasting was defined as ASM 2 standard deviations below the mean of a healthy reference group aged 18-40 years. Patients were divided into 3 groups according to the E/e' value as group A ≤8, group B 9-14, and group C ≥15. Muscle wasting was present in 19.7% of all patients. These patients performed worse in 6-MWT and showed lower absolute peak oxygen consumption. Values of muscle strength/ASM were associated with a better QoL. Logistic regression showed ASM to be independently associated with reduced

walking distance in 6-MWT adjusted for NYHA, height, left atrium diameter, ferritin and forced expiratory volume within the first second (FEV1). [59]

Further research was supported by a grant from the interdisciplinary center for clinical research at the University Hospital of Jena. We investigated here molecular, structural, and metabolic changes in skeletal muscle in patients with HF both HFpEF and HFrEF compared to healthy controls. Here is the summary:

2.1 Skeletal Muscle Function, Structure, and Metabolism in Patients with Heart Failure with Reduced Ejection Fraction and Heart Failure with Preserved Ejection Fraction

Reduced exercise capacity in patients with heart failure (HF) could be partially explained by skeletal muscle dysfunction. We compared skeletal muscle function, structure, and metabolism among clinically stable outpatients with HFpEF, HFrEF, and healthy controls (HC). Furthermore, the molecular, metabolic, and clinical profile of patients with reduced muscle endurance was described.

Fifty-five participants were recruited prospectively at the University Hospital Jena (17 HFpEF, 18 HFrEF, and 20 HC). All participants underwent echocardiography, CPET, 6-MWT, isokinetic muscle function, and skeletal muscle biopsies. Expression levels of fatty acid oxidation, glucose metabolism, atrophy genes, and proteins as well as inflammatory biomarkers were assessed. Mitochondria were evaluated using electron microscopy.

We found that patients with HFpEF demonstrated, compared with HFrEF and HC, reduced muscle strength (eccentric extension: 13.3±5.0 versus 18.0±5.9 versus 17.9±5.1 Nm/kg, P=0.04), elevated levels of MSTN-2 (myostatin-2), FBXO-32 (F-box only protein 32 [Atrogin1]) gene and protein, and smaller mitochondrial size (P<0.05). Mitochondrial function and fatty acid and glucose metabolism were impaired in HF patients compared with HC (P<0.05) **(Figure 2)**. In order to describe the molecular, metabolic, and clinical profile of patients with reduced muscle endurance, we divided the cohort into two groups according to the mean value of muscle endurance measured by extension of left leg with 180°.sec (Nm)/muscle mass of the left leg (kg) and found that reduced muscle endurance (< mean value of the cohort) was associated with elevated level of inflammatory biomarkers such as growth and differentiation factor 15 (GDF-15) and of atrophy-related protein (FBXO-32) measured both in western blot and with immunohistochemistry as well as with higher expressions of further atrophy-related genes. Peroxisome proliferator-activated receptor alpha (PPARα) and glucose-transporter-4 (GLUT4) as indicators of fatty acid oxidation and glucose metabolism

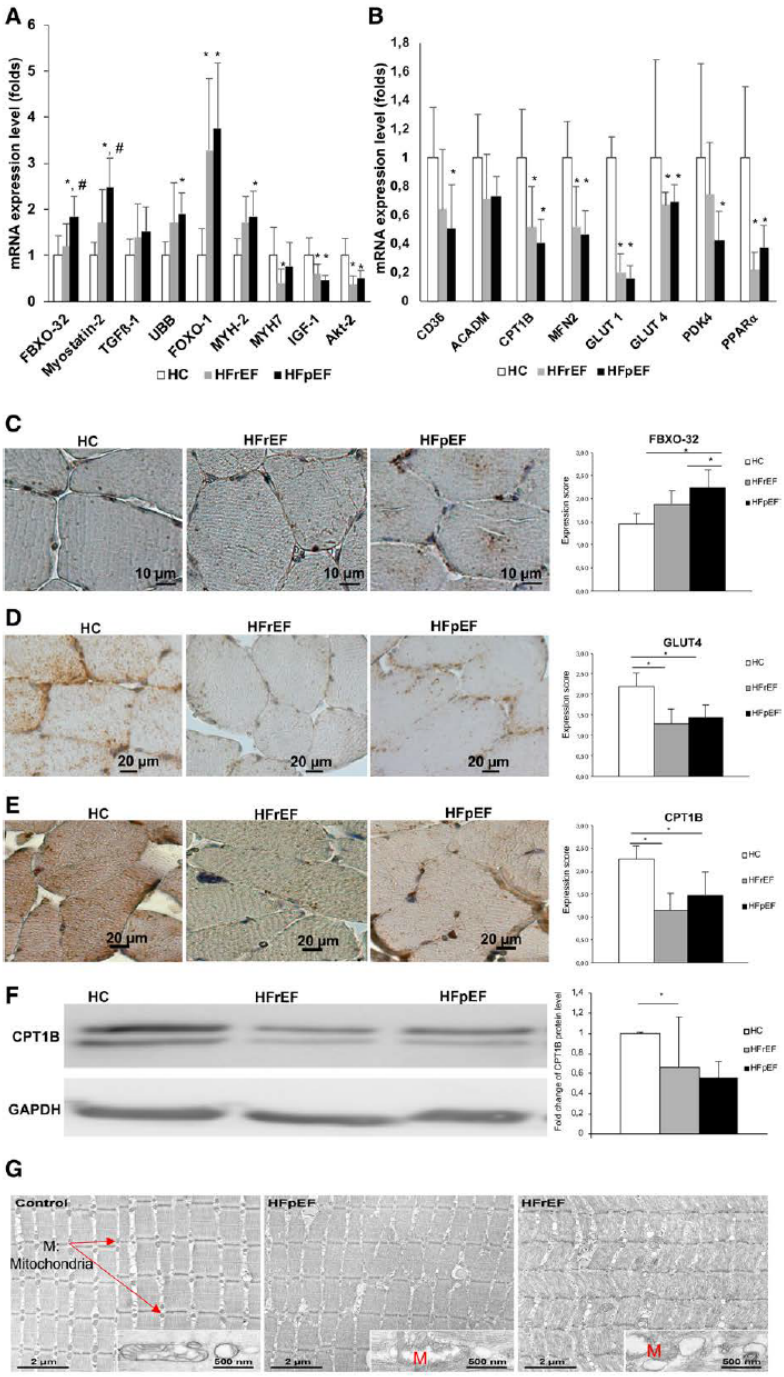
were reduced as well. Clinically, reduced muscle endurance was associated with reduced peak VO2 and walking distance in 6-MWT. In a multiple regression analysis, GDF-15, CPT1B (carnitine palmitoyltransferase IB)-protein and oral anticoagulation were independent factors for predicting reduced muscle endurance after adjusting for age (log10 GDF-15 [pg/mL] [B, −54.3 (95% CI, −106 to −2.00), P=0.043], log10 CPT1B per fold increase [B, 49.3 (95% CI, 1.90–96.77), P=0.042]; oral anticoagulation present [B, 44.8 (95% CI, 27.90–61.78), P<0.001]).

We concluded that patients with HFpEF have worse muscle function and predominant muscle atrophy compared with those with HFrEF and HC. Inflammatory biomarkers, fatty acid oxidation, and oral anticoagulation were independent factors for predicting reduced muscle endurance.

- Our study was the first study that described increased levels of atrophy genes and proteins as well as reduced mitochondrial size in skeletal muscle in stable outpatients with HFpEF compared with HFrEF and HC.
- We described the molecular, metabolic, and clinical profile of patients with reduced muscle endurance.
- Inflammation and fatty acid oxidation seem to be responsible for the reduced muscle endurance.

WHAT ARE THE CLINICAL IMPLICATIONS?

- Molecular, mitochondrial, and metabolic abnormalities in skeletal muscle in patients with HFpEF were associated clinically with reduced exercise capacity and reduced muscle function.
- This emphasizes the role of skeletal muscle on the reduced exercise capacity and dyspnea in patients with HFpEF.
- Focusing on endurance training or treating the above-mentioned abnormalities may lead to the improvement of the clinical condition of patients with heart failure with preserved ejection fraction.



Bekfani, T., et al., Skeletal Muscle Function, Structure, and Metabolism in Patients With Heart Failure With Reduced Ejection Fraction and Heart Failure With Preserved Ejection Fraction. Circ Heart Fail, 2020. 13(12): p. e007198.

Figure 2: Molecular and metabolic profile of skeletal muscle in patients with HFpEF compared to HFrEF and HC. **a:** Expression of catabolic (FBXO-32, Myostatin-2, UBB, FOXO, TGF-ß1, MYH 2) and anabolic genes (MYH 7, Akt-2 and IGF-1) in patients with HFpEF, HFrEF and HC. **b:** Expression of metabolic genes (fatty acid oxidation: CD36, ACADM, CTP1B, and MFN2, and glucose oxidation: GLUT1, GLUT4, PDK4, and PPARα) in skeletal muscle in HFpEF, HFrEF and HC. **c:** Protein level of FBXO-32 analyzed using immunohistochemistry in patients with HFpEF, HFrEF and HC. **d:** GLUT4-protein level in patients with HFpEF, HFrEF and in HC measured by immunohistochemistry. **e and f**: CPT1B-protein level in

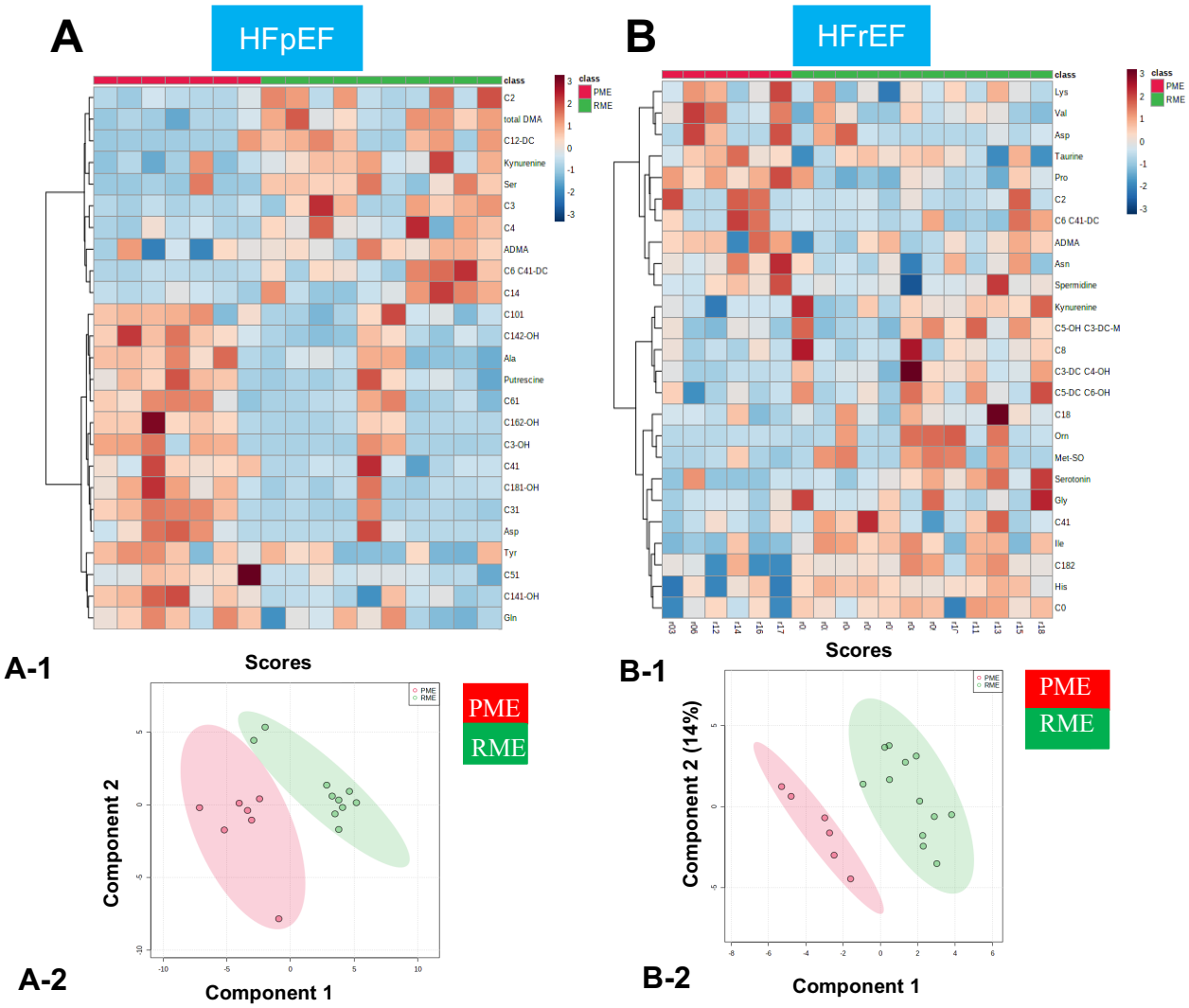
patients with HFpEF, HFrEF and in HC measured both by immunohistochemistry and western blot, respectively. **g:** Transmission electron microscopic images of mitochondria in skeletal muscle biopsies M: Mitochondria. ∗: $p<0.05$ in comparison between HFpEF/ HC and HFrEF/HC. #: $p<0.05$ in comparison between HFpEF/HFrEF
ACADM: medium-chain acyl-CoA dehydrogenase, Akt: protein kinase B, CD36: fatty acid translocase, CPT1B: carnitine palmitoyl transferase IB, FBXO-32: F-box only protein 32 (Atrogin1), FOXO: Forkhead box protein O, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, GLUT1,4: glucose-transporters 1,4, IGF-1: insulin-like growth factor 1, MFN2: mitofusin, MYH 7, 2: myosin fibers type I and type II, PDK4: pyruvate dehydrogenase kinase 4, PPAR α: peroxisome proliferator-activated receptor α TGF-ß: Transforming growth factor ß, UBB: ubiquitin B.

Metabolic changes taking place in patients with HF are reflected in various body fluids and tissues. As discussed above, metabolic, and structural perturbations in skeletal muscle have been found in patients with HF, both with HFpEF and HFrEF in association with reduced muscle endurance (RME) [65]. Thus, metabolomic analysis could support defining a distinct fingerprinting of various HF phenotypes and expand our knowledge on the pathogenic mechanisms underlying HF and its comorbidities such as sarcopenia or more mild forms such as reduced skeletal muscle function [66]. The next publication will be illustrated here:

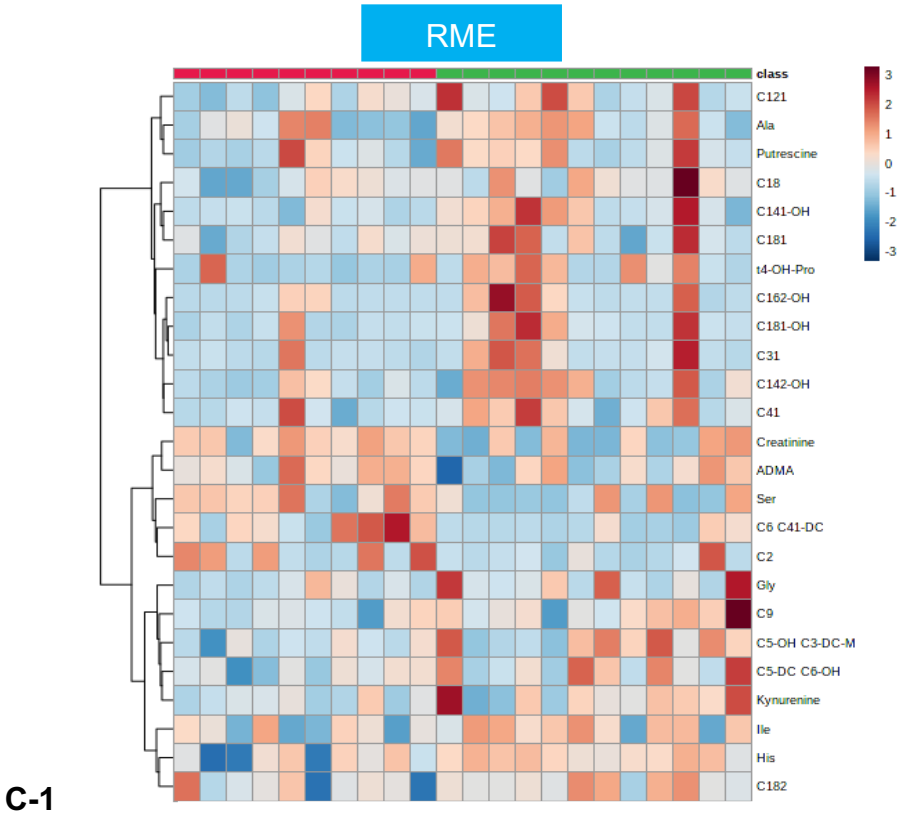
2.2 Metabolomic profiling in patients with heart failure and exercise intolerance: Kynurenine as a potential biomarker

In this study we aimed to create phenotypes for patients with RME and HFpEF compared to RME HFrEF according to their metabolomic profiles and to test the potential of Kynurenine (Kyn) as a marker for RME. The mean value of muscle endurance of quadriceps was used to divide the cohort into two groups (above or below the mean). RME was defined as muscle endurance <mean value of the cohort. Altogether, 18 HFrEF, 17 HFpEF, and 20 HC were prospectively included in the current study. The following tests were performed on all participants: isokinetic muscle function tests, echocardiography, spiroergometry, and various blood tests. Liquid chromatography tandem mass spectrometry was used to quantify metabolites in serum. Our results demonstrated that, except for aromatic and branched amino acids (AA), patients with HF showed reduced AAs compared to HC. Further perturbations were elevated concentrations of Kyn and acylcarnitines (ACs) in HFpEF and HFrEF patients ($p < 0.05$). While patients with HFpEF and RME presented with reduced concentrations of ACs (long- and medium-chains), those with HFrEF and RME had distorted AAs metabolism ($p < 0.05$) **(Figure 3)**. With an area under the curve (AUC) of 0.83, Kyn shows a potential as a

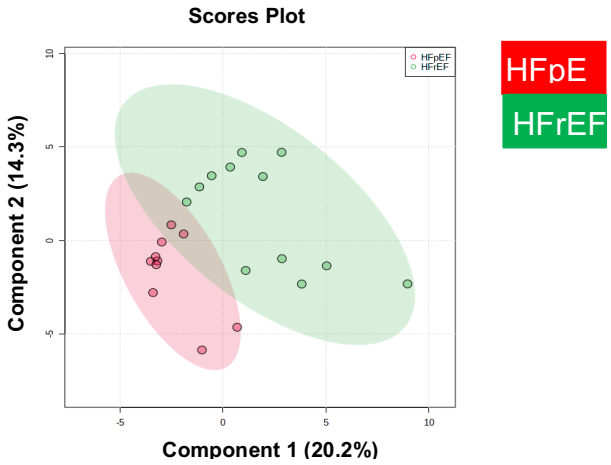
marker in HF and RME (specificity 70%, sensitivity 83%) **(Figure 4)**. In a multiple regression model consisting of short-chain-ACs, spermine, ornithine, glutamate, and Kyn, the latest was an independent predictor for RME (95% CI: −13.01, −3.30, B: −8.2 per 1 µM in-crease, p = 0.001). We concluded that RME in patients with HFpEF vs. HFrEF proved to have different metabolomic profiles suggesting varied pathophysiology. Kyn might be a promising biomarker for patients with HF and RME.



C



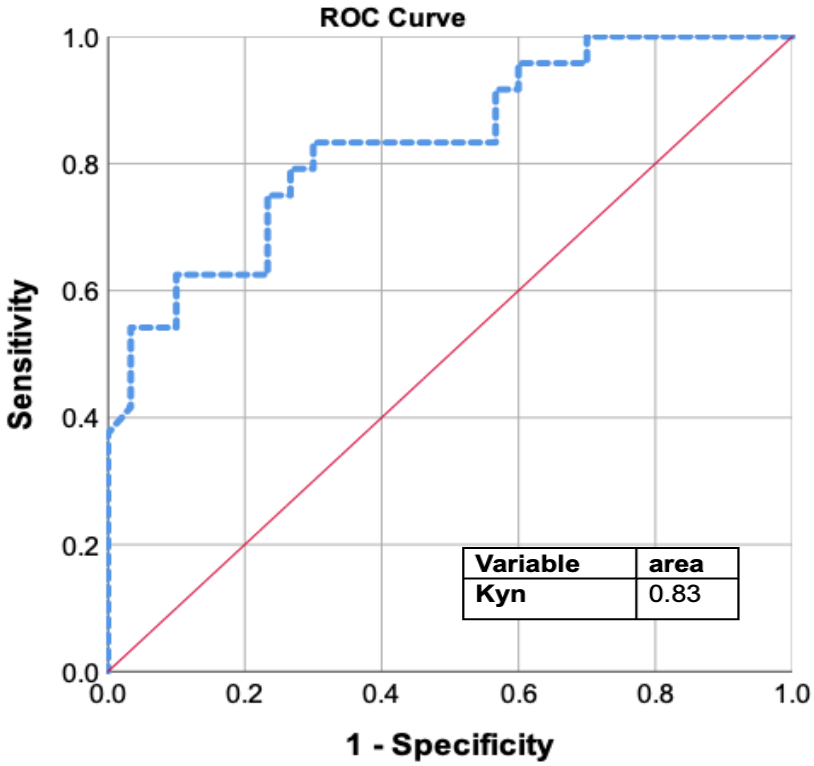
C-1



C-2

Bekfani T, et. al., Metabolomic profiling in patients with heart failure and exercise intolerance: Kynurenine as a potential biomarker. Cells. 2022 May 18;11(10):1674.

Figure 3: Metabolomic profiles of patients with reduced muscle endurance (RME) vs. preserved muscle endurance (PME) in HFpEF vs. HFrEF suggest different distorted metabolites and likely different mechanisms and metabolic pathways accompanying the RME. Visualization of the metabolomic profile using heat maps to show changes in the top 25 metabolite concentrations, and partial least squares-discriminant analysis (PLS-DA) among HFpEF (**A**, **A**-1 and **A**-2), HFrEF (**B**, **B**-1 and **B**-2), and in patients with RME and HFpEF vs. RME and HFrEF (**C**, **C**-1 and **C**-2), respectively.



Bekfani T, et. al., Metabolomic profiling in patients with heart failure and exercise intolerance: Kynurenine as a potential biomarker. Cells. 2022 May 18;11(10):1674.

Figure 4: The receiver operator characteristic (ROC) curve of Kynurenine to distinguish patients with RME of the left leg in flexion/muscle mass of the left leg. Area under the curve (AUC) is 0.83.

Further search on sensitive biomarkers for detecting RME showed that the left atrial function estimated with novel and modern methods such as left atrial strain (LAS) and left atrial emptying fraction (LAEF) could be promising in this respect, too:

2.3 Relation of left atrial function with exercise capacity and muscle endurance in patients with heart failure

As previously mentioned, patients with HFpEF and HFrEF present mainly with dyspnea and reduced exercise capacity [67]. These manifestations could be explained with central (cardiac) or peripheral (skeletal muscle) factors. One of the suggested mechanism is elevated left ventricular (LV) filling pressure [68]. Several studies have demonstrated that left atrial strain measured by 2-dimentional speckle tracking echocardiography (2D-STE) is a surrogate of elevated LV filling pressure [69-71]. Recently, left atrial function has gained attention due to the pivotal role of the left atrium in the resting and exercising cardiovascular system. Studies found a linear relationship between LA-function and maximal oxygen uptake (peak VO_2) during cardiopulmonary exercise testing (CPET) in different disease states such as HFpEF, diabetes mellitus, ischemic and dilated cardiomyopathies [72-76].

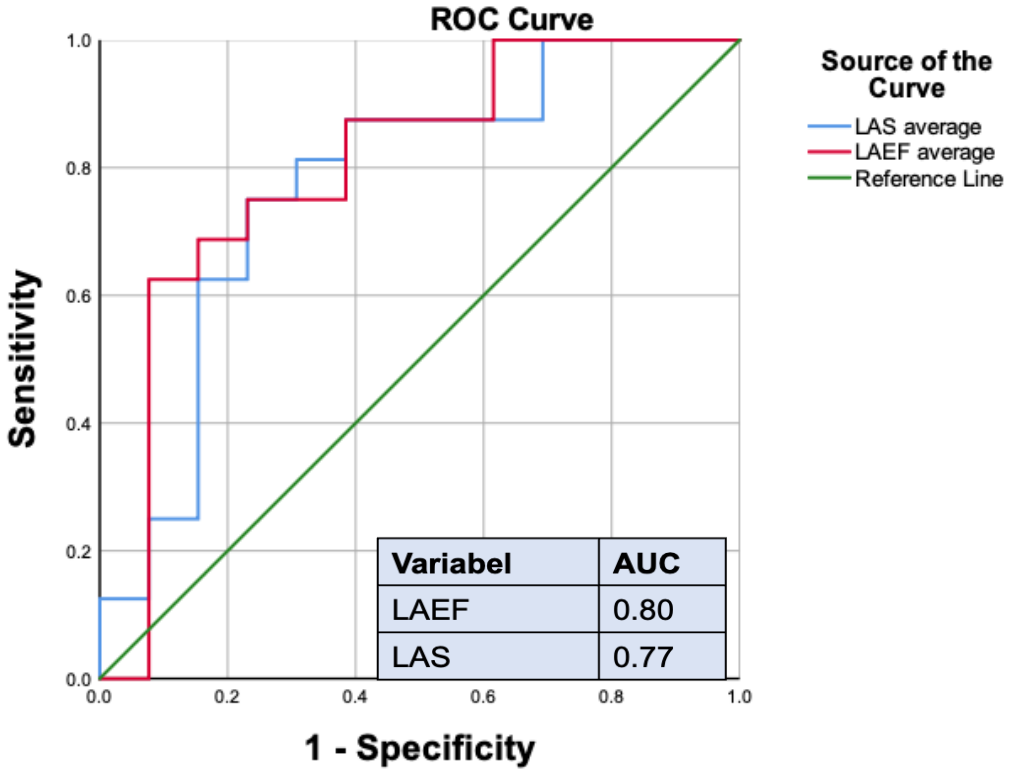
Similarly, reduced exercise capacity, measured as reduced peak VO_2, has been shown in several studies to be linked to peripheral factors such as skeletal muscle dysfunction both in patients with HFpEF and HFrEF [59, 64]. In fact, as discussed above about 20% of patients with HF suffer from skeletal muscle wasting, which is associated with reduced exercise and functional capacity (peak VO_2) [59, 64]. Additionally, as has been shown in a previous publication of our working group, molecular, mitochondrial, and metabolic abnormalities in skeletal muscle in patients with HFpEF and HFrEF were associated clinically with reduced exercise capacity and reduced muscle function [65].

As mentioned above, reduced peak VO_2 is associated with central and peripheral limitations. However, no direct relationship between central and peripheral factors could be proven yet. On the contrary, exercise training was shown to improve exercise tolerance in patients with HF independently of improving cardiac function measured as left ventricular ejection fraction [77]. One of the possible explanations for the failure in demonstrating a link between central and peripheral limitations of exercise capacity is likely not using more sensitive and novel cardiac measurements such as LAEF and LAS. LAS represents the severity of myocardial deformations. LAEF is calculated similarly to the left ventricle ejection fraction and both LAS and LAEF are surrogate novel parameters evaluating LA function and stiffness.

We hypothesized as the result to the multi-organ involvement in HF syndrome, an association between central and peripheral factors involved in the reduced exercise capacity in HF and searched for a sensitive cardiac parameter to demonstrate this relationship. Therefore, we investigated the association between LAS, LAEF, and left ventricular global strain (LVGLS) on one hand, and muscle endurance as surrogate of skeletal muscle function in patients with HFpEF, HFrEF and HC, on the other hand. We hypothesized that central novel parameters

(LAEF, LAS) are capable to detect the peripheral limiting factors (reduced skeletal muscle function).

We analyzed in this study echocardiographic measurements, CPET, and isokinetic muscle function in 55 subjects with HF and controls (17 HFpEF, 18 HFrEF and 20 HC) and found that patients with reduced LAEF showed reduced peak VO2: 14.3±3.5 vs. 18.5±3.5 ml/min/kg, p=0.003 and RME: 64.3±23.9 vs. 88.5±32.3 Nm/kg, p=0.028. Patients with reduced LAS showed similar results. Neither LVGLS nor left atrial volume index (LAVI) were associated with RME. The area under the curve of LAS and LAEF in patients with HF in association with RME were (0.76 vs.0.80) with 95% confidence interval (CI) (0.59-0.96, p=0.012 vs. 0.63-0.98, p=0.006, respectively) **(Figure 5)**. In a multiple linear regression, LAEF and working load measured during CPET (Watt) were independent factors for RME after adjusting for age, LVGLS and 6-MWT [LAEF (B: 0.09, 95% confidence interval (CI): 1.01; 1.18, p=0.024), working load (B: 0.05, 95% CI: 1.01; 1.08, p=0.006). Peak torque of the left leg was associated with E/LAS (E: early diastolic) in patients with HFpEF (r=-0.6, p=0.020). Endurance of the left leg was associated with LAEF (r=0.79, p=0.001) in patients with HFrEF. We concluded that LAS/LAEF are potential cardiac markers linking cardiac and peripheral limitations of exercise capacity. Thus, integrating LAS/LAEF in the evaluation of exercise intolerance in patients with HF could be useful.



Bekfani T, et. al., Relation of left atrial function with exercise capacity and muscle endurance in patients with heart failure. ESC Heart Fail. 2021 Dec;8(6):4528-4538.

Figure 5: The receiver operator characteristic (ROC) curve of left atrial emptying fraction (LAEF) and left atrial global longitudinal strain (LAGLS) to distinguish patients with reduced muscle endurance of left leg in extension/muscle mass of left leg (< mean value of the cohort of patients with HF). Area under the curve (AUC) is 0.80 vs. 0.76, respectively.

In a further publication, we showed a prognostic value of muscle wasting in patients with HF. Here are the details:

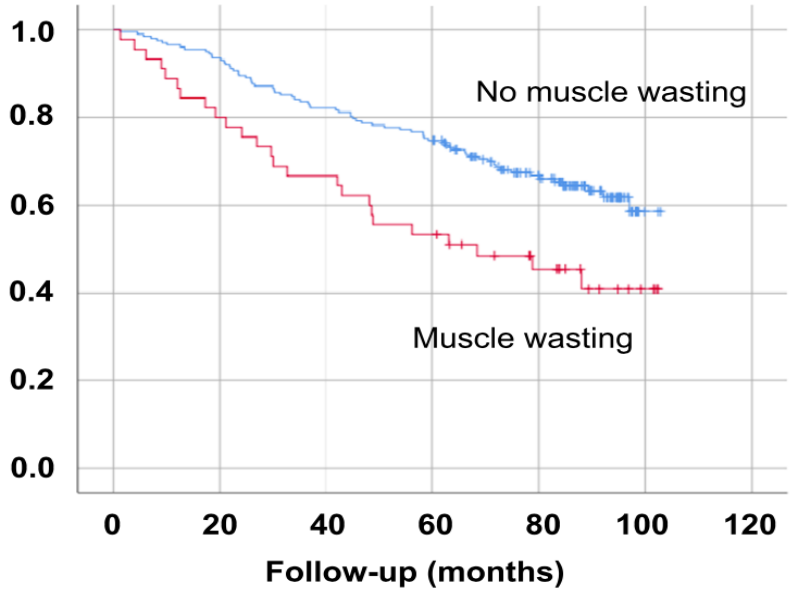
2.4 Muscle wasting as an independent predictor of survival in patients with chronic heart failure

It is known that skeletal muscle wasting is an extremely common feature in patients with HF, affecting approximately 20% of ambulatory patients with even higher values during acute decompensation. Its occurrence is associated with reduced exercise capacity, muscle strength, and QoL. We sought to investigate if the presence of muscle wasting carries prognostic information.

Two hundred sixty-eight ambulatory patients with HF (age 67.1 ± 10.9 years, NYHA class 2.3 ± 0.6, left ventricular ejection fraction 39 ± 13.3%, and 21% female) were prospectively enrolled as part of the SICA-HF. Muscle wasting, as assessed using DEXA, was present in 47 patients (17.5%).

Our results showed that during a mean follow-up of 67.2 ± 28.02 months, 95 patients (35.4%) died from any cause. After adjusting for age, NYHA class, left ventricular ejection fraction, creatinine, N-terminal pro-B-type natriuretic peptide, and iron deficiency, muscle wasting remained an independent predictor of death (hazard ratio 1.80, 95% confidence interval 1.01–3.19, P = 0.04) **(Figure 6)**. This effect was more pronounced in patients with HFrEF than in HFpEF.

We concluded that muscle wasting is an independent predictor of death in ambulatory patients with HF. This was the first study to describe the effect of muscle wasting on survival of patients with chronic HF. Clinical trials are needed to identify treatment approaches to this co-morbidity.



von Haehling S, Bekfani T, et. al., Muscle wasting as an independent predictor of survival in patients with chronic heart failure. J Cachexia Sarcopenia Muscle. 2020 Oct;11(5):1242-1249.

Figure 6: Kaplan-Meier survival curves by status of muscle wasting in the overall cohort.

Further entities of wasting disorders as co-morbidities of HF are ***cachexia and anorexia.***

Cachexia is , an involuntary loss of body mass, affects about 16% of patients with HF and is an independent predictor of worse overall prognosis [78].

The pathophysiology of cardiac cachexia is multifactorial in nature. Several mechanisms such as hormonal disturbances [79], overexpression of the pro-inflammatory cytokines, and malabsorption [80], and reduced food intake [81] have been reported. As previously shown, cachexia in HF is associated with right ventricular (RV) dysfunction rather than left ventricular (LV) impairment [82]. Adverse metabolic changes leading to cachexia may in part be attributed to venous congestion of the gastrointestinal system, which arises from RV failure. However,

the exact link between RV dysfunction and cachexia remains unknown. The aim of our next study was to evaluate signs of intestinal congestion and their relationship to cachexia in a cohort of ambulatory patients with HFrEF **(Figure 7)**.

Weight was measured in light clothes without shoes at baseline and follow-up. Weight change history up to 24 months prior to enrolment was documented based on medical reports or patients' personal statements. Cachexia was defined according to the current consensus-based diagnostic criteria [83]. Hence, the diagnosis was made in the presence of non-edematous, non-intentional weight loss of ≥5% over a period of at least 6 months. In addition, at least three of the following criteria had to be fulfilled: (i) decreased muscle strength, defined using a hand-grip strength test in the lowest tertile according to gender and age; (ii) fatigue; (iii) anorexia; (iv) lean tissue depletion, defined using the appendicular skeletal muscle index ,5.45 kg/m^2 for female and ,7.26 kg/m^2 for male patients [62], measured by DEXA, and (v) abnormal biochemistry, defined by elevated inflammatory markers [high-sensitivity C-reactive protein (hsCRP) > 5.0 mg/L] or anemia (hemoglobin ,12 g/dL). Here is the summery of this research work:

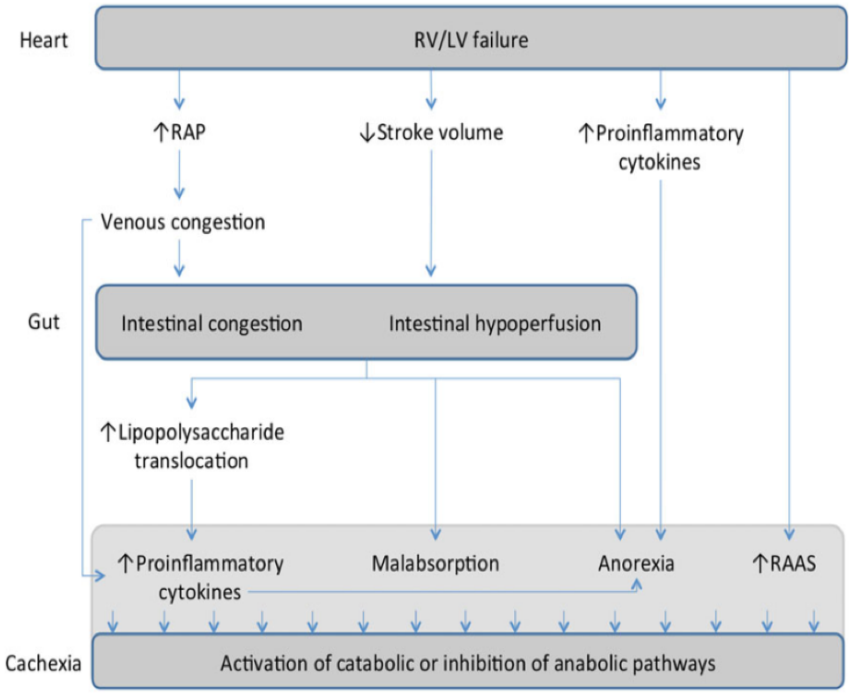
2.5 Intestinal congestion and right ventricular dysfunction: a link with appetite loss, inflammation, and cachexia in chronic heart failure

Mechanisms leading to cachexia in HF are not fully understood. We evaluated in this study signs of intestinal congestion in patients with chronic HF and their relationship with cachexia.

Of the 165 prospectively enrolled outpatients with left ventricular ejection fraction ≤40%, 29 (18%) were cachectic. Among echocardiographic parameters, the combination of right ventricular dysfunction and elevated right atrial pressure (RAP) provided the best discrimination between cachectic and non-cachectic patients [AUC 0.892, 95% CI: 0.832 – 0.936]. Cachectic patients, compared with non-cachectic, had higher prevalence of post-prandial fullness, appetite loss, and abdominal discomfort. Abdominal ultrasound showed a larger bowel wall thickness (BWT) in the entire colon and terminal ileum in cachectic than in non-cachectic patients. Bowel wall thickness correlated positively with gastrointestinal symptoms, hsCRP, RAP, and truncal fat-free mass, the latter serving as a marker of the fluid content. Logistic regression analysis showed that BWT was associated with cachexia, even after adjusting for cardiac function, inflammation, and stages of HF (odds ratio 1.4, 95% CI: 1.0 –

1.8; P-value=0.03). Among the cardiac parameters, only RAP remained significantly associated with cachexia after multivariable adjustment.

We concluded that cardiac cachexia was associated with intestinal congestion irrespective of HF stage and cardiac function. Gastrointestinal discomfort, appetite loss, and pro-inflammatory activation provide probable mechanisms by which intestinal congestion may trigger cardiac cachexia. However, our results are preliminary and larger studies are needed to clarify the intrinsic nature of this relationship.



Valentova M, Bekfani T, et. al., Intestinal congestion and right ventricular dysfunction: a link with appetite loss, inflammation, and cachexia in chronic heart failure. Eur Heart J. 2016 Jun 1;37(21):1684-91.

Figure 7: Possible mechanistic involvement of intestinal congestion in the pathophysiology of cardiac cachexia. Right ventricular failure and intestinal congestion may trigger malabsorption of nutrients, loss of appetite, and increased translocation of bacterial lipopolysaccharide. All these factors, along with long-term activation of renin-angiotensin-aldosterone system, may lead to cachexia.

Intestinal congestion, cachexia, and HF might be associated with anorexia. Currently, it is acknowledged that the term malnutrition suggests a nutritional problem or failure, according to the combination of loss of food intake with age-related changes and other HF-related co-morbidities. Anorexia, defined as the loss of desire to eat, is a multifactorial process, despite caloric deprivation is frequently seen in HF patients [84].

2.6 Anorexia, functional capacity, and clinical outcome in patients with chronic heart failure

Anorexia is also reported as an independent and strong predictor of morbidity and mortality among patients in various clinical settings [85]. In oncology patients, the anorexia-cachexia syndrome is diagnosed as weight loss accompanied by anorexia, associated with impaired functional capacity and worse outcomes [86]. Despite the growing importance of anorexia, cachexia, and functional capacity for QoL, there is still a lack of information explaining whether and how they relate to each other in patients with HF [87]. The reasons for anorexia are multifactorial. For example, drug therapy, hepatic or gastrointestinal congestion and gastrointestinal dysfunction, and metabolic disturbance could affect appetite. Therefore, the determinants of anorexia and the influence of anorexia on functional capacity and outcome are of special interests in patients with HF.

Evaluation of anorexia

A single-item measure was used to assess the presence of anorexia in all participants [88]. Subjects were asked to answer the question 'Do you have appetite loss?' (Rated as 6-point Likert scale 0 = not at all, 1 = very rarely, 2 = rarely, 3 = occasionally, 4 = frequently, and 5 = very frequently with higher number indicating increased frequency of anorexia). A cut-off value ≥ 1 on the anorexia scale was used to define anorexia [88].

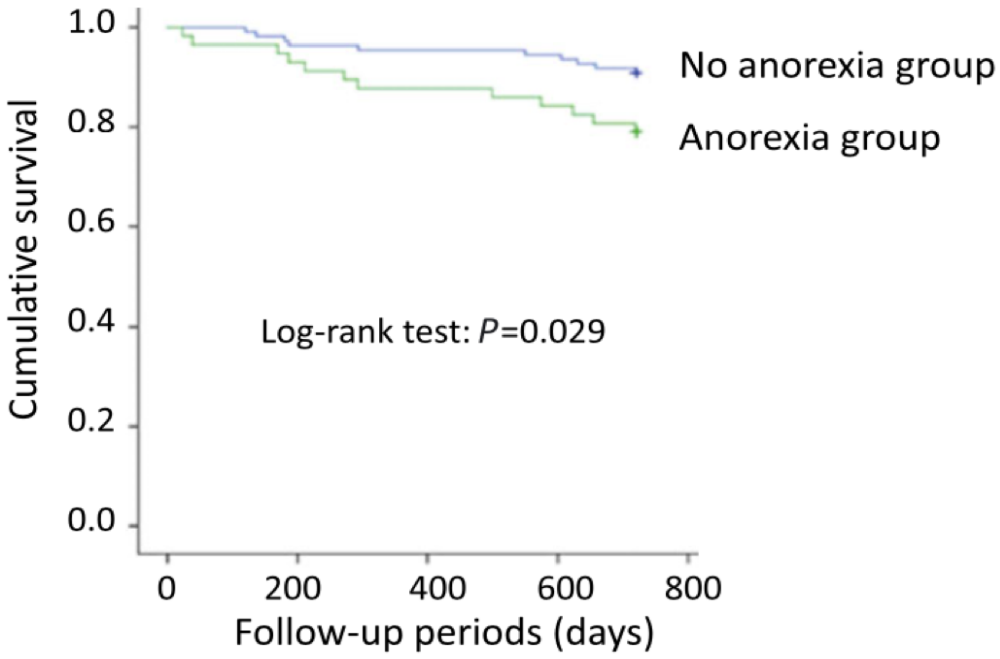
We aimed in this study to assess determinants of anorexia in patients with HF and aimed to further elucidate the association between anorexia, functional capacity, and outcomes in affected patients.

We assessed in this study anorexia status among 166 patients with HF (25 females, 66 ± 12 years) who participated in SICA-HF.

Functional capacity was assessed as peak oxygen uptake (peak VO_2), 6 min walk test, and short physical performance battery test. A total of 57 patients (34%) reported any anorexia and these patients showed lower values of peak VO_2, 6 min walk distance, and short physical performance battery score (all $P < 0.05$). Using multivariate analysis adjusting for clinically important factors, only hsCRP [OR 1.24, P = 0.04], use of loop diuretics (OR 5.76, P = 0.03),

and the presence of cachexia (OR 2.53, P = 0.04) remained independent predictors of anorexia. A total of 22 patients (13%) died during a mean follow-up of 22.5 ± 5.1 months. Kaplan-Meier curves for cumulative survival showed that those patients with anorexia presented higher mortality (Log-rank test P = 0.03) **(Figure 8)**.

We concluded that inflammation, use of loop diuretics, and cachexia are associated with an increased likelihood of anorexia in patients with HF, and patients with anorexia showed impaired functional capacity and poor outcomes.



Saitoh M, Bekfani T, et. al., Anorexia, functional capacity, and clinical outcome in patients with chronic heart failure: results from the Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF). ESC Heart Fail. 2017 Nov;4(4):448-457.

Figure 8: Kaplan-Meier survival curve.

Moving to another common morbidity in HF, namely iron deficiency (ID) and anemia, we analyzed the prevalence of ID in HFpEF and its relation to reduced exercise capacity, muscle strength and QoL.

3. IRON DEFICIENCY AND ANEMIA

Anemia is a known co-morbidity across the spectrum of HF with either preserved or reduced ejection fraction [89]. Anemia is associated with worse prognosis and reduced functional capacity in this group of patients [89].

Iron plays a key role in oxygen uptake, transport, and storage, as well as oxidative metabolism in the skeletal muscle; it is also involved in erythropoiesis [90]. However, erythropoiesis remains undisturbed until late in the course of iron depletion [91]. ID is defined as ferritin <100 microg per liter or ferritin between 100-299 microg per liter, if transferrin saturation <20% [39]. It has been reported that ID with or without anemia impairs the aerobic performance and leads to fatigue and exercise intolerance [92]. It is also known that the intravenous repletion—as opposed to oral administration [93]—of iron in patients with HFrEF improves functional capacity, symptoms, and QoL and may be associated with reduced hospitalization rates for worsening in HF [39].

ID is an extremely common nutritional disorder that affects up to 2 billion people worldwide [91], and it has recently been reported as a frequent co-morbidity in stable HFrEF patients [94]. Furthermore, ID—but not anemia—was found to be an independent predictor of worse outcome in HFrEF patients [95].

However, less is known about ID in HFpEF patients. Thus, we performed the following multicenter, prospective, cross-sectional study:

3.1 Iron deficiency in patients with heart failure with preserved ejection fraction and its association with reduced exercise capacity, muscle strength, and quality of life

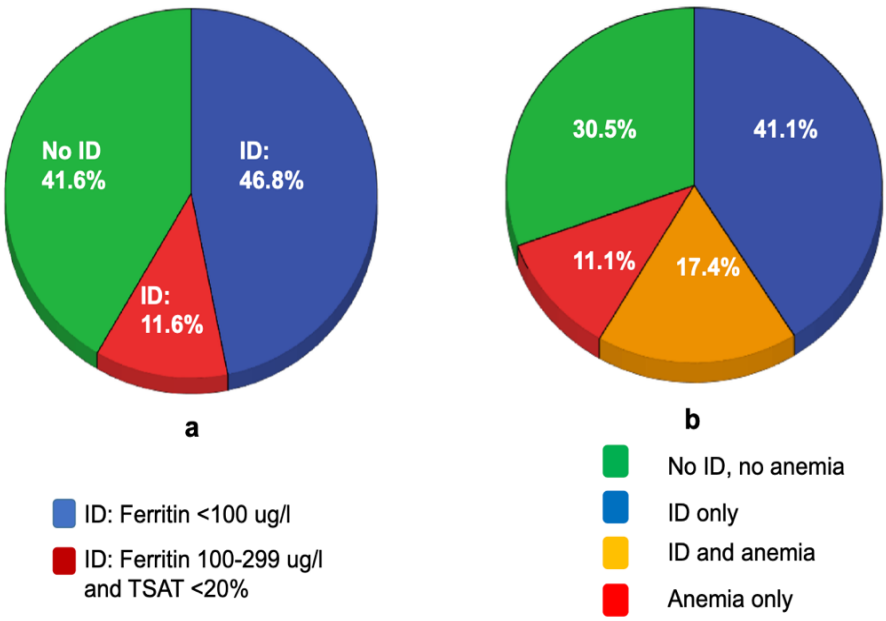
The prevalence of ID in outpatients with HFpEF and its relation to exercise capacity and QoL is unknown.

One hundred nintety symptomatic outpatients with HFpEF (LVEF 58±7%; age 71±9 years; NYHA 2.4±0.5; BMI 31±6kg/m2) were enrolled as part of SICA-HF in Germany, England and Slovenia. ID was defined as ferritin < 100 or 100–299 μg/L with transferrin saturation (TSAT)

< 20%. Anemia was defined as hemoglobin (Hb) < 13 g/dL in men, < 12 g/dL in women. Low ferritin-ID was defined as ferritin < 100 µg/L. Patients were divided into 3 groups according to E/e′ at echocardiography: E/e′ ≤ 8; E/e′ 9–14; E/e′ ≥ 15. All patients underwent echocardiography, CPET, 6-MWT, and QoL assessment using the EQ5D questionnaire.

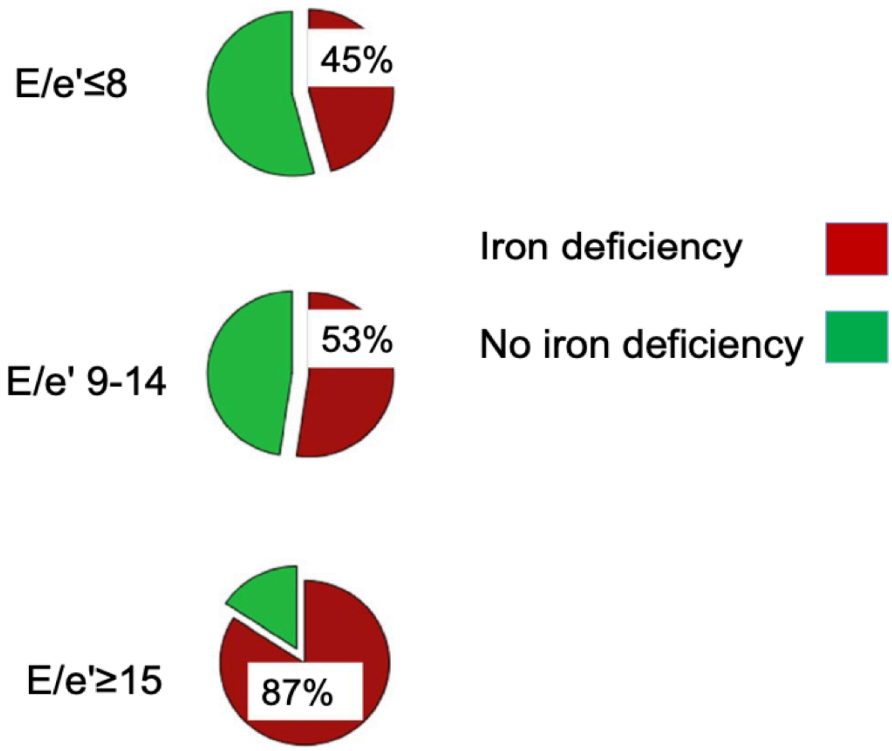
We found that overall, 111 patients (58.4%) showed ID with 89 having low ferritin-ID (46.84%). Seventy eight (41.1%) patients had isolated ID without anemia and 54 patients showed anemia (28.4%) **(Figure 9).** ID was more prevalent in patients with more severe diastolic dysfunction: E/e′ ≤ 8: 44.8% vs. E/e′: 9–14: 53.2% vs. E/e′ ≥ 15: 86.5% (p = 0.0004) **(Figure 10).** Patients with ID performed worse during the 6MWT (420±137 vs. 344±124 m; p=0.008) and had worse exercise time in CPET (645±168 vs. 538±178 s, p=0.03). Patients with low ferritin-ID had lower QoL compared to those without ID (p = 0.03).

We concluded that ID is a frequent co-morbidity in HFpEF and is associated with reduced exercise capacity and QoL. Its prevalence increases with increasing severity of diastolic dysfunction.



Bekfani T, et. al., Iron deficiency in patients with heart failure with preserved ejection fraction and its association with reduced exercise capacity, muscle strength and quality of life. Clin Res Cardiol. 2019 Feb;108(2):203-211.

Figure 9**: a:** Prevalence of Iron deficiency both with Ferritin <100ug/ml and ferritin between 100-299 ug/ml with TSAT <20% in patients with HFpEF. **b:** Prevalence of both ID and anemia in patients with HFpEF.



Bekfani T, et. al., Iron deficiency in patients with heart failure with preserved ejection fraction and its association with reduced exercise capacity, muscle strength and quality of life. Clin Res Cardiol. 2019 Feb;108(2):203-211.

Figure 10: Prevalence of ID in groups with different severities of HFpEF.

A further aspect of comorbidities accompanying HF are psychological disorders. Patients with HF, especially those with HFpEF, suffer from exercise intolerance that not only impairs physical activity but also mental, psychological, and social life aspects in these patients [96-98]. Psychological and mental disorders such as depression and anxiety are common in both HFpEF and HFrEF [99, 100] and have been proven to be independently associated with higher mortality and readmission rates [98, 101]. Depression prevalence for example in patients with HF is 15-40% and it increases the risk for morbidity and mortality [102].

4. CENTRAL NERVOUS SYSTEM (DEPRESSION AND ANXIETY)

Recent studies focused on peripheral factors such as skeletal muscle in explaining the reduced exercise capacity, dyspnea, and QoL [59, 64]. One study in animal models showed a link between skeletal muscle dysfunction and depression [103]. Another group demonstrated that exercise training in HFpEF improves physical, psychological, and social components of

QoL [98]. A further study in acute decompensated HF showed an association between physical function, cognitive dysfunction, and QoL [104].

However, a systematic comprehensive comparison among clinically stable outpatients with HFpEF, HFrEF, and age-matched non-HF controls regarding QoL, depression, and anxiety and the relationship to coordination capacity and inflammatory biomarkers is still missing. We hypothesized that patients with HFpEF have worse QoL and increased prevalence of anxiety and depression compared to those with HFrEF and non-HF controls. Additionally, we investigated the link between QoL, depression, coordination capacity, inflammatory process, and muscle function in these patients. Here is the summery of our study:

4.1 Psychosocial factors, mental health, and coordination capacity in patients with HFpEF compared to HFrEF

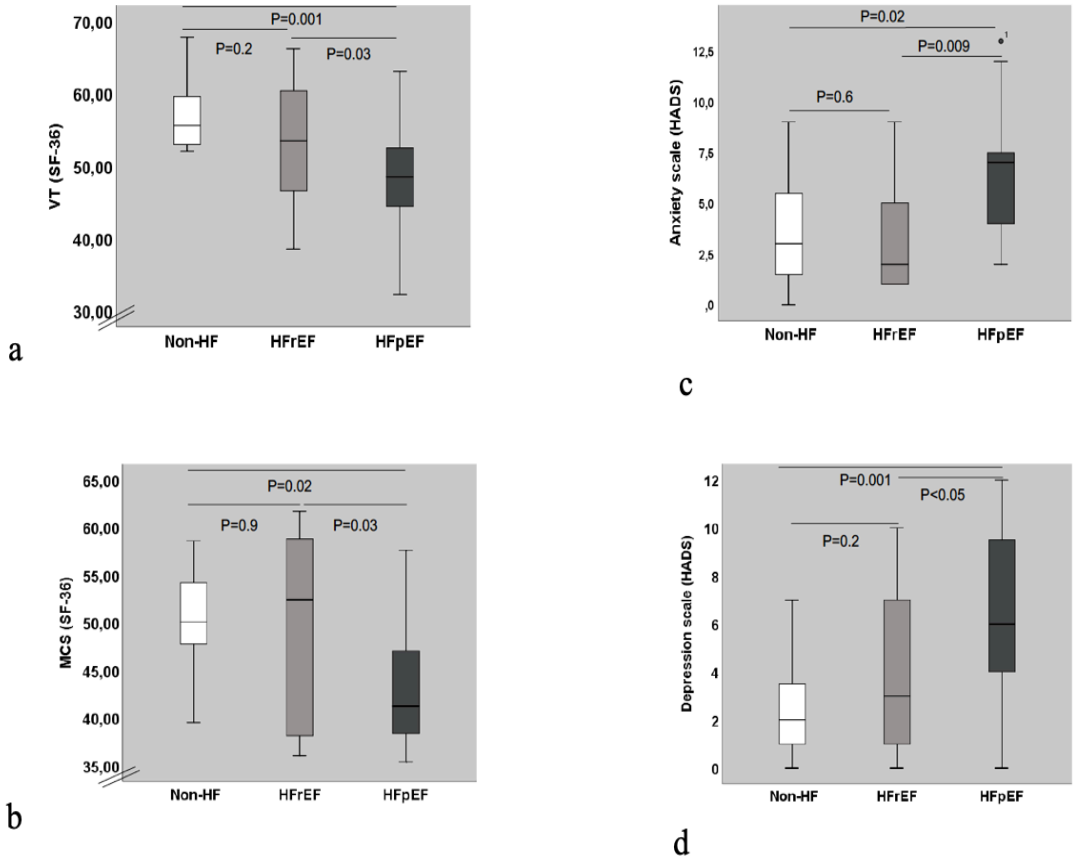
We aimed in this study to compare QoL, depression, and anxiety scores among outpatients with HFpEF and HFrEF and non-HF controls (non-HF) and its relationship to coordination capacity.

Fifty-five participants were recruited prospectively (17 HFpEF, 18 HFrEF, 20 non-HF). All participants underwent echocardiography, CPET, 10-meter walking test (10-MWT), isokinetic muscle function and coordination tests, and QoL- assessments using the short form of health survey (SF-36), and hospital anxiety and depression scale (HADS). Furthermore, inflammatory biomarkers such as GDF-15 were assessed.

We found that patients with HFpEF showed compared to HFrEF and non-HF reduced QoL [mental component score (MCS): 43.6±7.1 vs. 50.2±10.0 vs. 50.5±5.0, p=0.03), vitality (VT): 47.5±8.4 vs. 53.6±8.6 vs. 57.1±5.2, p=0.004), and elevated anxiety (6.5±3.2 vs. 3.3±2.8 vs. 3.8±2. 8, p=0.02) and depression scores (6.5 [3.5-10.0] vs. 3.0 [1.0-6.5] vs. 2.0 [0.75-3.0], p=0.01)] **(Figure 11)**. HFpEF and HFrEF patients showed reduced coordination capacity compared to non-HF ($p<0.05$). Patients with an elevated depression score showed reduced (MCS: 41.2±4.8 vs. 49.2±9.6, p=0.04), and (VT: 44.8±7.5 vs. 52.2±8.5, p=0.04). Furthermore, we found that peak torque of knee in eccentric extension and after adjusting to sex was significantly lower in patients with HFpEF and HFrEF than in non-HF controls (151±50.8 vs. 187±39.7 vs. 220±42.1 Nm/kg, p=0.02). Peak torque of right knee in eccentric flexion was associated with peak torque of right knee in concentric flexion was associated with (r=0.7, $p<0.0001$) and inversely with balance coordination capacity (walking backward on 15 cm wide line: r=-0.4, p=0.04). These correlations remained unchanged after adjusting to sex.

In a logistic regression, the presence of depression score ≥8 remained an independent factor for predicting reduced coordination capacity after adjusting for peak VO_2, GDF-15, 10-MWT, physical component score (PCS), and peak torque of the leg [OR: 0.1, 95% CI: 0.004-0.626, p=0.02].

We concluded that outpatients with HFpEF had worse QoL and higher anxiety and depression scores compared to HFrEF and non-HF. Depression is associated with reduced QoL and is an independent predictor for reduced coordination capacity.



Bekfani T, et. al., Psychosocial factors, mental health, and coordination capacity in patients with heart failure with preserved ejection fraction compared with heart failure with reduced ejection fraction. ESC Heart Fail. 2021 Aug;8(4):3268-3278.

Figure 11: Comparison of quality of life between HFpEF, HFrEF patients and non-HF controls. a: Vitality (VT) as part of the SF-36-questionnaire. b: Mental health component summery (MCS) as part of the SF-36-questionnaire. c: Anxiety scale as part of the HADS-questionnaire. d: Depression scale as part of the HADS-questionnaire. HADS: Hospital anxiety and depression scale, HFpEF: Heart failure with preserved ejection fraction, HFrEF: Heart failure with reduced ejection fraction, MCS: Mental health component summery, non-HF: non-heart failure, SF-36: short form of health survey, VT: Vitality.

As previously extensively discussed, HF is a syndrome affecting almost all organs and systems of the body. However, these organs have not being investigated systematically in a routine manner. Liver diseases are only one further example:

5. LIVER FIBROSIS

Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease which encompasses nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NASH promotes progressive liver fibrosis and thus, can cause cirrhosis. In patients with NAFLD, advanced liver fibrosis as measured by validated scoring systems is linked to worse clinical outcomes including all-cause mortality [105], and cardiovascular disease is one of the leading causes of mortality [106]. The prevalence of NAFLD in HFpEF is high (>25%) based on several small observational studies [107, 108]. Both HFpEF and NAFLD are characterized by metabolic dysregulation. Accumulating evidence suggests that NAFLD, independently of other established risk factors for HF, is associated with HFpEF/diastolic dysfunction [109-111], and further, that liver disease could precede HFpEF onset [112]. NAFLD has also been shown to be associated with changes in myocardial structure and function over time, although obesity appears to account for much of this association [113].

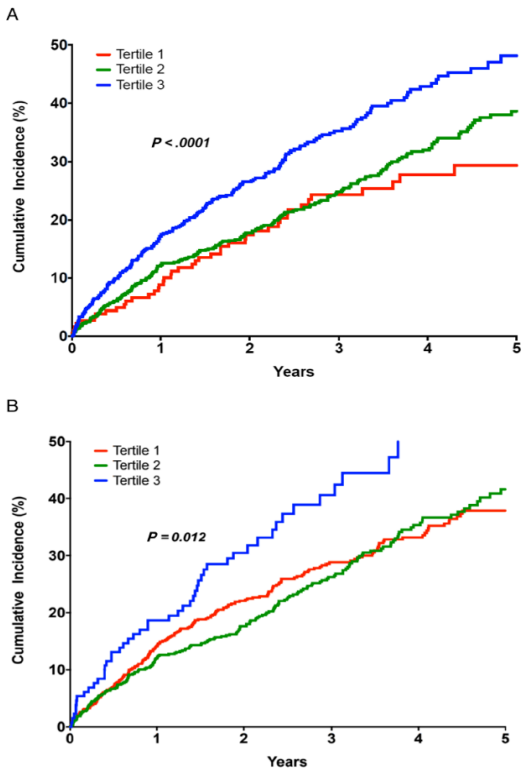
Despite this known overlap in pathology, liver disease is often not assessed in HFpEF clinical practice and clinical trials. Therefore, the prevalence and implications of liver disease in a contemporary cohort of HFpEF are unclear. This analysis aimed to address these gaps in knowledge by describing the prevalence of liver fibrosis, as assessed using surrogate biomarkers, and the association of such biomarkers in predicting clinical outcomes in patients with HFpEF.

5.1 Association of liver fibrosis risk scores with clinical outcomes in patients with heart failure with preserved ejection fraction: findings from TOPCAT

Patients with HFpEF from TOPCAT Americas were included in the analysis. The non-alcoholic fatty liver disease fibrosis score (NFS) and fibrosis-4 (FIB-4) scores were calculated using a combination of clinical characteristics and laboratory parameters. Risk of advanced fibrosis was classified as low, intermediate, and high. For the 1423 patients with sufficient data, we used Cox regression analysis to test the association between the risk of fibrosis severity and the

combined primary endpoint of all cardiovascular death, aborted cardiac arrest, and hospitalization for HF. Advanced fibrosis, as determined by high fibrosis scores, was present in 37.57% by the NFS and 8.02% by the FIB-4. Higher risk of advanced hepatic fibrosis was associated with older age. In unadjusted models, the risk of advanced fibrosis was associated with the primary cardiovascular outcome [NFS high vs. low, hazard ratio (HR) 1.709 (95% CI 1.238–2.358, P = 0.0011) and FIB-4 high vs. low, HR 1.561 (95% CI 1.139–2.140, P = 0.0056)]. After multivariable adjustment, this association was diminished [NFS high vs. low, HR 1.349 (95% CI 0.938–1.939, P = 0.1064) and FIB-4 high vs. low, HR 1.415 (95% CI 0.995–2.010, P = 0.0531)] **(Figure 12).**

In conclusion, our study suggests that advanced liver fibrosis, as estimated by fibrosis risk scores, is not uncommon in patients with HFpEF, and there appears to be a limited independent association between liver fibrosis risk scores and clinical outcomes related to HF events.



Peters AE, Bekfani T, et. al., Association of liver fibrosis risk scores with clinical outcomes in patients with heart failure with preserved ejection fraction: findings from TOPCAT. ESC Heart Fail. 2021 Apr;8(2):842-848.

Figure 12: (A) Kaplan-Meier curves for primary outcome split into non-alcoholic fatty liver disease fibrosis score (NFS) groups and (B) Kaplan-Meier curves for primary outcome split by fibrosis-4 (FIB-4) Groups 1-3.

The last forgotten comorbidity that we investigated was sleep-disordered breathing (SDB).

6. SLEEP-DISRODERED BREATHING

SDB is one of the most common comorbidities in patients with HF. Its prevalence ranges between 55-80% [114]. SDB refers to a big variety of sleep related breathing disorders including obstructive sleep apnea (OSA) and central sleep apnea (CSA). However, the awareness of its co-existence in these patients is low and there are unfortunately no routinely used screening programs for SDB in patients with HF. This could be partially a result of the high expenses spent on polysomnography, the gold standard to establish the diagnosis of SDB, and the limited availability of sleep labs even in developed countries. However, screening for SDB is possible with an ambulatory device that can be used in the patients' home (polygraphy). The analysis of sleep studies distinguishes between two types of SDB: 1. CSA and 2. OSA. However, both types can overlap and present as mixed sleep apnea [115].

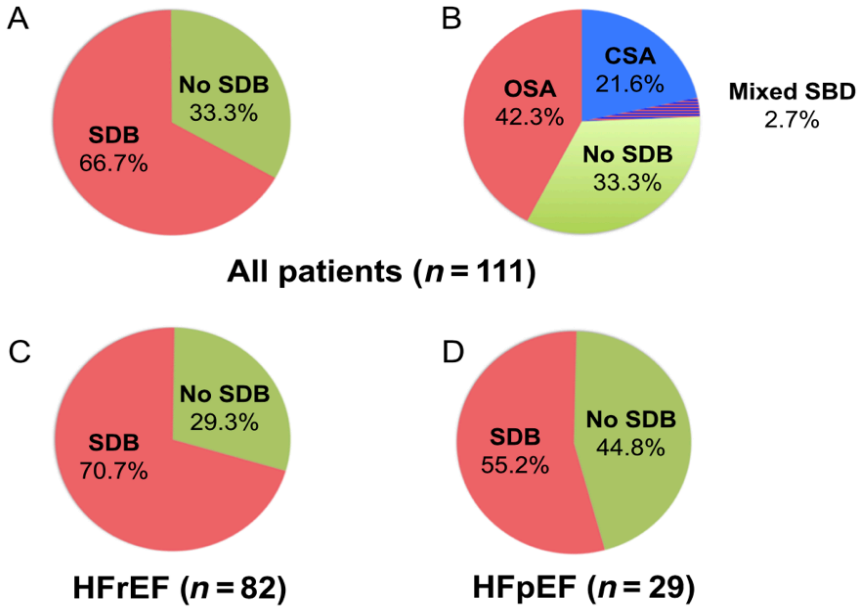
Changes in body composition among patients with SDB have been described in the general population in a limited number of smaller studies [116]. Two methods are helpful in this regard: DEXA and bioelectrical impedance analysis (BIA). Whilst DEXA uses small amounts of radiation, the principle of BIA depends on an electric current that flows at different rates encountering different electrical resistance values in the body due to the different electrical conduction properties of water, fat, or bone [117]. The water is localized in two compartments: extra-cellular water (ECW) and intracellular water (ICW). Fat tissue allows for significantly less conductibility than water, muscle, or bone [117]. Body composition in patients with SDB and the differences between CSA and OSA are not well investigated and understood yet in the field of HF. As a result of increased sympathetic activation, SDB may have significant impact on body composition or on the occurrence of muscle wasting. Indeed, there are few, if any, studies, that described the relationship between body composition on one hand and exercise capacity and the severity of HF and SDB in these patients on the other hand.

We sought to investigate the characteristic differences in body composition among patients with SDB of either CSA or OSA type and to investigate the co-existence of muscle wasting (sarcopenia) in this group of patients. The prevalence of SDB in clinically stable outpatients with HF was evaluated using a portable screening device. Furthermore, we aimed to study the

relationship between the total amount of water as a sign of preclinical congestion and exercise capacity and the severity of HF and SDB.

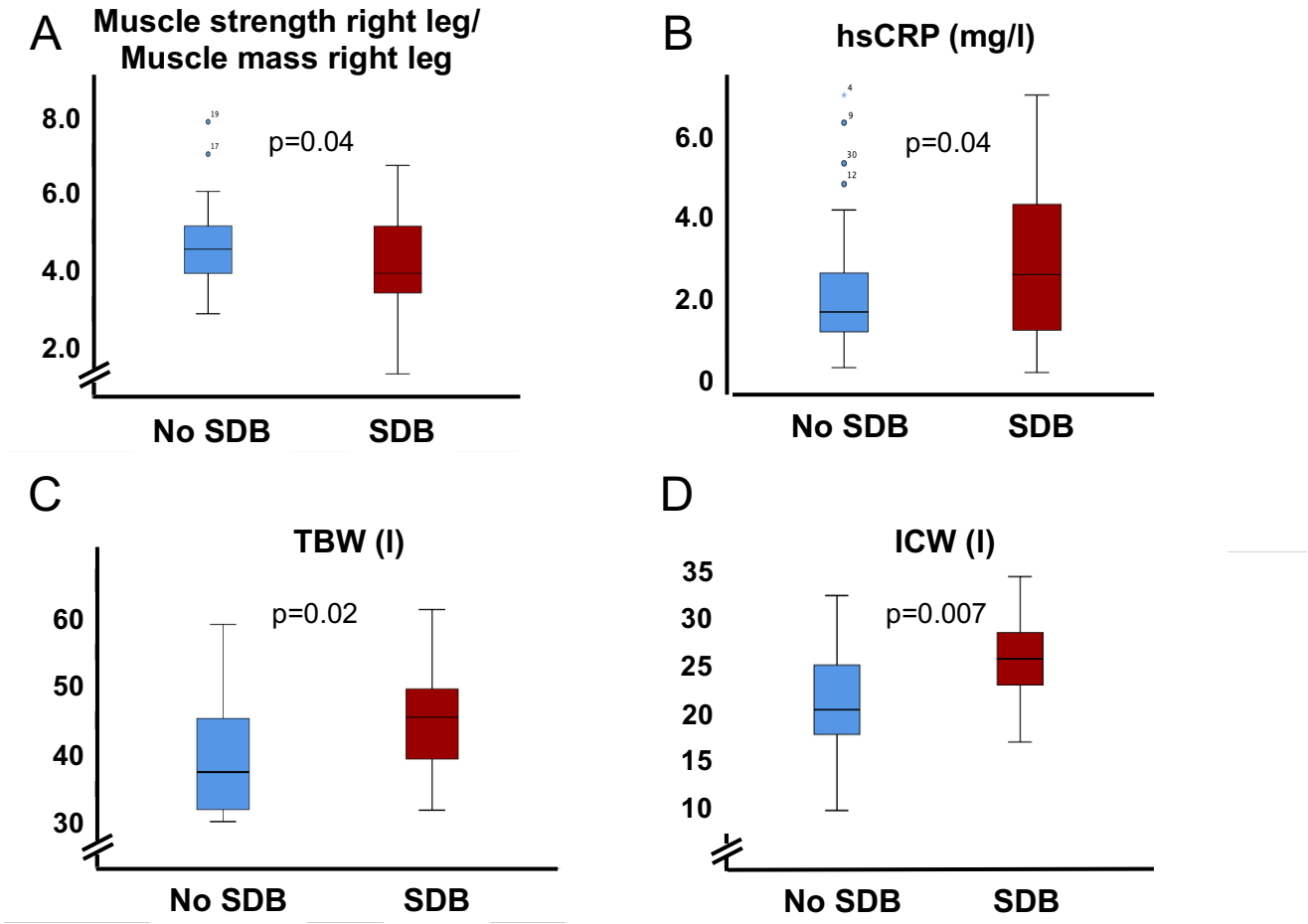
6.1 Heart failure and sleep-disordered breathing: susceptibility to muscle wasting and preclinical congestion

We studied 111 outpatients with stable HF who were enrolled into the SICA-HF study. Echocardiography, short physical performance battery (SPPB), CPET, DEXA, BIA, tests of muscle strength, and polygraphy were performed. SDB was defined as apnoea/hypopnoea index (AHI) >5 per hour of sleep. CSA and OSA were defined as AHI >50% of central or obstructive origin, respectively. A total of 74 patients (66.7%) had any form of SDB [CSA (24 patients, 32.4%), OSA (47 patients, 63.5%)] **(Figure 13)**. Patients with SDB showed increased muscle weakness (chair stand), reduced muscle strength, and lower values of SPPB score ($P < 0.05$). Patients with SDB did not show overt clinical signs of cardiac decompensation compared with those without SDB ($P > 0.05$) but had increased amounts of water (total body water, intracellular, and extracellular) measured using BIA ($P < 0.05$) **(Figure 14)**. Increased amounts of total body water were associated with the severity of SDB and inversely with muscle strength and exercise capacity measured by anaerobic threshold ($P < 0.05$). Altogether, 17 patients had muscle wasting. Of these, 11 (65%) patients had SDB (statistically not significant).
Our conclusion was that SDB is highly prevalent in patients with HF. Patients with SDB have lower muscle strength and tend to be more susceptible to preclinical congestion.



Bekfani T, et. al., Heart failure and sleep-disordered breathing: susceptibility to reduced muscle strength and preclinical congestion (SICA-HF cohort). ESC Heart Fail. 2020 Oct;7(5):2063-2070.

Figure 13: (A, B) The prevalence of sleep-disordered breathing (SDB) in patients with heart failure. (C) The prevalence of SDB in heart failure with reduced ejection fraction (HFrEF) patients. (D) The prevalence of SDB in heart failure with preserved ejection fraction (HFpEF) patients.



Bekfani T, et. al., Heart failure and sleep-disordered breathing: susceptibility to reduced muscle strength and preclinical congestion (SICA-HF cohort). ESC Heart Fail. 2020 Oct;7(5):2063-2070.

Figure 14: Comparison between HF-patients with and without SDB regarding: A. Muscle strength right leg/muscle mass right leg, B. High-sensitivity C-reactive protein (hsCRP), C. Total body water (TBW), D. Intracellular water (ICW).

7. DISCUSSION

In our study **"Skeletal muscle function, structure, and metabolism in patients with HFrEF and HFpEF"**, we found that HFpEF patients have reduced muscle function, reduced mitochondrial size, and elevated levels of atrophy-related genes and protein (FBXO-32, MSTN-2) compared to HFrEF and HC. Perturbations in fatty acid oxidation and glucose metabolism as well as reduced mitochondrial volume density were noted in HFpEF and HFrEF patients compared to HC. These changes were associated with reduced exercise capacity. The results remained unchanged after adjusting for age and gender.

By dividing the cohort into two groups according to the mean value of muscle endurance, we described for the first time the molecular, metabolic, and clinical profile of reduced muscle endurance and found that patients with reduced muscle endurance showed reduced gene expression of slow muscle fibers myosin (MYH 7), elevated levels of atrophy genes and proteins (TGF- ß1, FOXO-1, UBB, TRIM63, FBXO-32), and lysosomal gene (CTSL). Furthermore, the expression of PPARα and GLUT4 were reduced in these patients. This was associated with an elevated inflammatory biomarker level (GDF-15) and reduced values of peak VO_2 and 6MWT. There was neither an association with age nor with physical daily activity estimated in the QoL-questionnaire.

Several factors play an important role in triggering muscle wasting and atrophy in patients with HF. Proinflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6 are some examples of these factors [118]. Muscle atrophy as such occurs as a result of imbalance between protein synthesis and degradation. One of the most important protein degradation pathways is activation of the ubiquitin-proteasome system (UPS). This enzymatic process involves binding a small signaling protein (ubiquitin) in a mono- or a polyubiquitination to the target protein [119, 120]. Polyubiquitination UPS is required to degrade target proteins. The rate-limiting enzymes in this process are the E3 ubiquitin-protein ligases [119, 121]. The most commonly associated E3-ubiquitin-protein ligases are TRIM-63 (also known as MURF1) and FBXO-32 (MAFBX or atrogin 1). It is known from several atrophy models that both TRIM-63 and FBXO-32 are specifically induced in muscle [122]. The expression of both of these genes is regulated primarily by the transcription factors nuclear factor-κB (NF-κB) and FOXO family members [123]. The latter factors are in turn induced by the elevated pro-inflammatory cytokines (TNF) in patients with HF [124]. GDF-15 is a novel inflammatory biomarker that has been shown to be elevated in patients with HF [125]. However, its relation to skeletal muscle function was not tested yet. We showed that GDF-15 is elevated in patients with reduced muscle

endurance. In line with this, we found in our current study that patients with HFpEF showed elevated FBXO-32, and those with reduced muscle endurance showed elevated TRIM63 and FBXO-32.

Additionally, we found that patients, who were treated with aldosterone antagonists (spironolactone/eplerenone) showed reduced level of FBXO-32 and had at the same time less muscle atrophy. It is known that aldosterone antagonists play a positive role in cardiac remodeling in patients with HF. Whether this group of medication plays similarly a positive remodeling role in skeletal muscle needs to be further investigated. This could open a new horizon in the treatment of patients with HF and muscle atrophy.

A further aspect of our study was to investigate the metabolic function of skeletal muscle. We investigated here the changes of mitochondrial volume density, size and number, and the expression level of genes responsible for mitochondrial dynamics (MFN 1,2) and uncoupling (UCP3). Additionally, the fatty acid and glucose oxidation processes were measured in both HF-patients and HC. Mitochondria are ubiquitous membrane-bound organelles that are a defining feature of the eukaryotic cell. The organelle is comprised of a soluble matrix surrounded by a double membrane, an ion impermeable inner membrane, and a permeable outer membrane. Early biochemists recognized the importance of mitochondria as the sites of aerobic oxidation of metabolic fuels. It is now well established that mitochondria contribute to many important functions including fatty acid oxidation [126].

We found significantly reduced number, size, and volume density of mitochondria and disturbed glucose and fatty acid oxidation both in patients with HFpEF and HFrEF compared to HC. Decreased mitochondrial number and size was shown in previous studies to be associated with reduced exercise capacity in patients with HF [119, 127]. We confirmed this association and found additionally a profound reduction in the size of mitochondria in HFpEF patients compared to HFrEF and HC and a strong association between mitochondrial size and the expression of rate-limiting genes and proteins of fatty acid and glucose oxidation as well as with ventilatory threshold and peak VO_2, muscle strength, and inflammatory biomarker.

Mitochondria are dynamically regulated and undergo repeated rounds of fusion and fission, even moving around the cell [128]. This highly regulated phenomenon is known as mitochondrial dynamics and is mediated by members of the mitofusin family (mitofusin 1 and mitofusin 2, also known as MFN1 and MFN2) [129].

Fatty acid oxidation is a multistep process to produce energy through breakdown of fatty acids. The entrance of free fatty acids into the cells is primarily protein-dependent using protein transporters on the cell surface (FAT/CD36) [130]. Through esterification, acyl-CoA is formed,

specifically for long-chain fatty acids. Carnitine palmitoyltransferase 1 (CPT1) conversion of the long-chain acyl-CoA to long-chain acylcarnitine is a rate-limiting process for fatty acid moiety and its transport across the inner mitochondrial membrane [130]. Furthermore, ACADM is an important gene responsible for providing instruction to produce the enzyme Acyl-coenzyme A dehydrogenase, which degrades medium-chain fatty acids [130]. Additionally, PPARα is a key regulator of fatty acid oxidation in skeletal muscle [131]. All of these rate-limiting steps of fatty acid oxidation showed abnormalities in skeletal muscle in HF compared to HC.

A multiple linear regression analysis showed that the inflammatory biomarker (GDF-15) and the rate-limiting protein in fatty acid oxidation (CPT1B) as well as OAC-therapy remain independent factors for reduced muscle endurance after adjusting to age.

In conclusion, we found accentuated muscle dysfunction in patients with HFpEF compared to those with HFrEF and HC both clinically and on molecular basis. A multi-level and multi-pathway disorders of protein synthesis and protein degradation, mitochondrial, and metabolic disorders in skeletal muscle of patients with HF especially in those with HFpEF were shown. Additionally, muscle endurance was associated with elevated inflammatory biomarkers and reduced fatty acid oxidation. As a result, setting the priority on modifying the inflammatory process and optimizing peripheral fatty acid oxidation could be reasonable first steps in developing therapies for skeletal muscle dysfunction in HF, especially in patients with HFpEF.

It is assumed that skeletal muscle as one of the main energy depots of the human muscle is lost earlier than other tissues like adipose tissue or even bone mineral density [132]. However, the latter two are also affected in patients with manifest cachexia in advanced HF in whom tissue loss is so advanced that affected patients are losing body weight. One of the main challenges remains, however, to identify patients early and before weight loss becomes apparent. Our study **"Muscle wasting as an independent predictor of survival in patients with chronic heart failure"** underscores the assumption that the loss of skeletal muscle without manifest weight loss already identifies patients at increased risk of death. Indeed, cardiac cachexia had already been described as an independent predictor of death more than 20 years ago, but it is now becoming clear that skeletal muscle plays a pivotal role in this setting, because patients who lose muscle are unable to exercise to a sufficient degree as highlighted by low QoL. Apart from those reason named-above as factors supporting catabolic process, further factors and mechanisms could be involved in the reduced muscle function such as peripheral blood flow [133], abnormal ergoreflex physiology [134], and altered nutritional intake [135], but probably

also iron deficiency and anemia [136]. In particular, iron deficiency has been shown to have effects in skeletal muscle, because enzymes of the mitochondrial electron transport chain are iron-dependent, and recent data show that iron administration can help to improve phosphocreatine recovery in the muscle [137].

Taken together, muscle wasting beyond the cut-offs defined to identify sarcopenia, identifies a large proportion of patients with HF who have low muscle strength, QoL, exercise capacity, and who are likely to become frail in that they may be at increased risk of falling, risk of fractures and hospitalizations. Our data show that these patients are also at 2-fold increased risk of death.

As discussed above, detecting muscle wasting earlier is very important. DEXA is the currently used method to establish the diagnosis. However, DEXA scanning is not routinely available in many hospitals. Thus, there is a need for new biomarkers that are capable to detect these changes early enough and in an easier way. Metabolomic analysis is an emerging discipline that has the potential to characterize patients with different diseases, such as HF, according to their metabolomic profiles [17]. We described in **the article "Metabolomic profiling in patients with heart failure and exercise intolerance: Kynurenine as a potential biomarker**" a distinct metabolomic profile in patients with HFrEF and HFpEF compared to HC. Additionally, metabolomic profiles of patients with HF and RME were defined. These patients had reduced concentrations of Amino acids and elevated concentrations of Kyn and GDF-15. Clinically, this was associated with reduced 6-MWT.
To our best knowledge, the findings of our current study report for the first time distinct metabolomic abnormalities in HFpEF patients with RME (reduced unsaturated-, medium-chain-, long-chain-ACs, and medium-/long-chain ACs, reduced Alanine, and elevated Kyn) compared to HFpEF patients with preserved muscle endurance (PME). Furthermore, RME was associated with different metabolomic perturbations in HFpEF (disorders in fatty acid uptake and oxidation and mitochondrial metabolism) than in HFrEF (reduced concentrations of AAs) patients. These findings might focus a special spot of light on the distorted metabolic pathways and the associated pathophysiology of the altered function and structure of the skeletal muscle in patients with HFpEF and HFrEF. As a result, this might have direct therapeutic implications.

Contrary to what is expected, studies in obese patients with diabetes and in those on high-fat feeding have shown increased rates of β-oxidation in skeletal muscle. This was mainly

explained by “incomplete” fat oxidation, where fatty acids enter the mitochondria but are not completely degraded [138]. However, such an analysis has not been performed yet considering the skeletal muscle function of patients with HFpEF and RME. Though our analysis was not performed directly in skeletal muscle tissues, we described for the first time the metabolomic profile of HF patients with RME.

Furthermore, activated β-oxidative genes do not always lead to increased expression of downstream metabolic pathways such as the tricarboxylic acid cycle (TCA cycle), also known as the Krebs cycle, and the electron transport chain. Instead, in some cases, this was associated with decreased expression levels of *PGC-1a*, a transcriptional coactivator that is responsible for mitochondrial biogenesis and function [139]. This all is in line with our above-mentioned results and with the reduced levels of C0/(C16 + C18) in patients with HFpEF and RME, which was proven to be a surrogate of increased activity of *CPT1B* [140]. This all supports the mitochondrial dysfunction in HF with RME and shows that ACs apparently enter the mitochondria but are likely not appropriately metabolized or are “incompletely oxidized” in the mitochondria. This is in line with our previous results showing reduced levels of *MFN-2* in skeletal muscle of patients with HF and RME [65]. Our results need to be confirmed in further and larger prospective studies.

CPT1B intermediates the most rate limiting step of fatty oxidation: the conversion of fatty acyl-CoA esters into fatty acylcarnitine derivatives, which enter the mitochondria where *CPTB2* convert them back into fatty acyl-CoA in preparation for further mitochondrial β-oxidation [141]. Defects of the β-oxidation that suppresses the oxidation of long-chain fatty acids lead to the accumulation of mitochondrial long-chain acyl-CoAs, which, in turn, makes the mitochondria ineffective. On the other hand, impaired long-chain fatty acid β-oxidation produces decreases in short- and medium-chain acyl-CoAs and finally reduced concentrations of short- and medium-chain AC derivatives as a result of abnormal downstream cycles of long fatty acids of β-oxidation. Therefore, reductions in short-, medium-, and medium-/long-chain AC ratios in the circulation might be a manifestation of impairments in mitochondrial long-chain fatty acid oxidation rates. If fatty acid oxidation is weakened according to inhibition of mitochondrial fatty acid uptake, decreases in short-, medium-, and long-chain ACs could be observed [141]. Our findings show in HFpEF patients and RME an increased fatty acid oxidation rate and, at the same time, give some hints about mitochondrial dysfunction likely in other proteins than *CPT1B*. The causality between RME and fatty acid metabolism needs to be further clarified in future research.

A further abnormality was noted in HFpEF patients with RME that might enhance the reduced energy production in these patients. Alanine concentration was significantly more reduced compared to HFpEF patients with PME. Alanine is exported from skeletal muscles to the liver to support hepatic gluconeogenesis [141].
Patients with HFrEF and RME compared to HFrEF and PME showed mainly reduced concentrations of AAs without any apparent alterations in fatty acid oxidation.

A further important finding of our current study was showing elevated concentrations of Kyn in the serum of patients with HF (HFrEF and HFpEF) and RME. It has been shown recently that Kyn is elevated in patients with HFrEF [142]. We confirmed these results and extended this knowledge to patients with HFpEF. Furthermore, in a multiple regression model consisting of short-chain-ACs, spermine, ornithine, glutamate, and Kyn, the latest was an independent predictor for RME. Additionally, we found a strong association between biogenic amines such as Kyn and inflammatory factors like GDF-15.
Tryptophan (Trp) metabolism in physiologic conditions takes place in liver cells using tryptophan 2,3-dioxygenase [143]. However, during inflammatory processes or oxidative stress, such as in HF, Trp is metabolized by indoleamine-pyrrole 2,3-dioxygenase (IDO) in other cell types, mainly in the blood and lymphoid tissues [144]. In the latest conditions, several products, such as Kyn, 3-hydroxykynurenine, anthranilic acid, 3-hydroxyanthranilic acid, kynurenic acid, and quinolinic acid, result from the degradation of Trp. [145]. IDO-related pathways and the resulting elevated Kyn were proven to be involved in cardiometabolic diseases such as metabolic syndrome or atherosclerosis [146, 147]. Recently, Kyn was found to lead to cardiomyocyte apoptosis after myocardial infarct in a mice model through the production of reactive oxygen species [148]. Parallel to that, a recent study showed that the distorted Trp-metabolism pathway and the resulting elevated Kyn concentrations were associated with atherosclerosis and myocardial infarct [146]. Our findings are in line with the above-mentioned studies, showing an association between the elevated Kyn concentrations in serum and the impaired function of the skeletal muscle (RME) in patients with HF. This might be suggestive of a generalized Kyn-related pathophysiology in inflammatory processes such as HF that seems to take place in several tissues, such as heart muscle and skeletal muscle. Our results need to be further investigated in prospective studies or in animal models with HF and RME to prove the causality.

Searching for further biomarkers for detecting RME, we showed in a further paper "**Relation of left atrial function with exercise capacity and muscle endurance in patients with heart failure**" the link between central (LAEF, LAS) and peripheral factors (skeletal muscle function) involved in the pathophysiology of reduced exercise capacity in patients with HF. We showed a high AUC of both LAEF and LAS in association with RME. In a multiple linear regression, LAEF and working load measured during CPET (watt) were independent factors for predicting RME after adjusting for age, LVGLS and 6MWT.

To describe the profile of patients with reduced LAEF, we divided the cohort into two groups according to the mean value of LAEF and found that patients with reduced LAEF have reduced exercise capacity measured as peak VO2 and elevated VE/VCO2, as well as reduced muscle function measured as peak torque and muscle endurance of legs both in flexion and extension. Similar results were shown by dividing the cohort according to the mean value of LAS. In other words, we showed for the first time a relationship between central and peripheral limitations of exercise capacity in patients with HF both with HFpEF and HFrEF. Neither LVGLS nor LAVI were as sensitive and did not show any relation to muscle endurance. Peak torque of the left leg in patients with HFpEF was inversely associated with E/LAS. The elevated novel LA filling index (E/LAS ratio) was recently shown to be an effective and useful parameter to determine the elevated LV filling pressure in patients with HFpEF [25]. Accordingly, our findings show that HFpEF patients with elevated LV filling pressure (elevated E/LAS ratio) correlated with reduced muscle strength of legs.

Patients with HF suffer mainly from dyspnea and reduced exercise capacity measured in the CPET as reduced peak VO_2 [67, 149, 150]. The pathophysiology beyond dyspnea and exercise intolerance in patients with HF is multifactorial and includes both central (cardiac) and peripheral (skeletal muscle) factors [59, 151, 152]. A link between cardiac and muscular function contributing to the reduced peak VO_2 in HF, as a result to the systemic involvement of HF, is expected. In other words, we hypothesized that central novel parameters (LAEF, LAS) are capable to detect the peripheral limiting factors (reduced skeletal muscle function).

The role of peripheral factors such as skeletal muscle mass and function in explaining the reduced exercise capacity in patients with HF have been shown in several studies [59, 64, 65].

Centrally, elevated filling pressure of the left ventricle was suggested as an important mechanism in explaining dyspnea and reduced exercise capacity [67, 151]. LAS is a surrogate of elevated left ventricular filling pressure and an indicator of cardiovascular performance

through regulating pulmonary venous return and LV filling [71, 153]. Recent studies and guidelines have defined the normal values of LAEF (>48%) and LAS (>26%) [69, 154].
Additionally, recent studies showed a link between LA function measured by 2D-STE and reduced exercise capacity with CPET [72, 74, 155]. The latest relationship could be explained by the anatomical location and function of the LA. The LA functions as a reservoir during systole, conduit during early diastole and a blood pump in the late diastole [156]. The harmony of all these three phases is very important to keep the atrioventricular coupling intact during exercise and therefore maintaining the best possible cardiac output and exercise capacity. One of the adaptative mechanisms of the LA to maintain the atrioventricular coupling is to increase LA volume through LA dilation and keeping as a result the LV filing pressure optimally as low as possible [157], which leads finally to increase LA volume and reduce LA function. HF-guidelines recommend the evaluation of LAVI [158]. However, the relationship between LA function using 2D-STE and exercise capacity (peak VO_2) is stronger than LAVI [74]. Furthermore, LA dysfunction was documented in patients with hypertension or diabetes even with normal LA size [159]. Recently, LA function has also been shown to be an independent predictor for HF-hospitalization [160]. *Frydas et al.* found recently in an analysis in 300 patients with HF that LAS is more sensitive than LAVI in detecting LA impairment in HF and that LAS is superior to LAEF, LAVI, or E/e′ in predicting the presence elevated LV filling pressures. Furthermore, the diagnostic value of LAS was independent from LVEF [161].
In spite of the strong correlation shown in our results between LVGLS and LAS/LAEF, LVGLS failed to predict the reduced exercise capacity measured evaluated both as peak VO_2 or as RME. Lundberg et al. found in a simultaneous echocardiography and invasive hemodynamic measurements that LAS correlates with pulmonary capillary wedge pressure (PCWP) but not LVGLS [162]. This all emphasizes the importance of using LAS/LAEF and not LAVI or LVGLS in evaluating LA-function and left ventricular filling pressure. This was explained by the fact that LAS quantifies mechanical events at the LA level associated with PCWP, as opposed to LVGLS, which might better reflect left ventricular end diastolic pressure. Furthermore, previous experimental studies have shown distinct cellular responses with more pronounced pro-fibrotic changes detected in the LA as compared to the LV wall [163], that supports the diagnostic importance of LAS/LAEF independent of the LVGLS.

Another related comorbidity of HF is cachexia, which was already defined above. In our publication **“Intestinal congestion and right ventricular dysfunction: a link with appetite**

loss, inflammation, and cachexia in chronic heart failure", we found that cardiac cachexia is associated with intestinal congestion in stable systolic HF patients, even after adjusting for other covariates involved in wasting. This corresponded with elevated right atrial pressure due to systolic RV dysfunction, both found in the majority of cachectic patients, indicating a hemodynamic link between congestive RV dysfunction and intestinal edema. Larger BWT correlated with abdominal discomfort, appetite loss, postprandial fullness, and inflammation, which were also found to be highly prevalent in cachectic patients and might mechanistically explain the association between intestinal congestion and cachexia. These findings are important, as the results from treatment trials in patients with cardiac cachexia have been discouraging so far, thus questioning our understanding of this complex comorbidity.

In the current study, cachexia was stronger linked to elevated RAP than isolated RV dysfunction, suggesting an interaction between venous congestion (such as in HF) and wasting. Of note, not all patients with RV dysfunction had a concomitant increase in RAP. These patients were considerably less likely to be cachectic or to lose weight during the follow-up compared with those with elevated RAP. On the other hand, a subset of patients with a formally normal RV function had elevated RAP and was more likely to have cachexia. Thus, it appears that elevated RAP is a more reliable parameter for stratifying patients with advanced HF regarding the risk for cachexia than systolic RV function, not to mention LV function alone. However, patients with reduced RV function and elevated RAP had the highest prevalence of cachexia, highlighting the hemodynamic dependence between RV and RA pressures. This pressure overload is further transmitted to the venous system resulting in splanchnic congestion and, in some cases, consecutive intestinal edema. This explains our finding of larger BWT in patients with elevated RAP but the absence of the former in patients with normal RAP.

Splanchnic congestion was a typical finding in cachectic patients as shown by larger BWT and higher truncal water content compared with non-cachectic patients. Larger BWT was associated with appetite loss, postprandial fullness, and inflammation. These abnormalities, along with malabsorption, which may also result from venous congestion of the bowel wall, have pro-catabolic effects and offer a mechanistically plausible explanation for the observed association between intestinal congestion and cachexia. In our study, more than two-thirds of cachectic patients suffered from appetite loss and postprandial fullness. These two factors were related to intestinal congestion and can further worsen reduced food intake that was shown in general HF population accelerating cachexia [164]. A second mechanism, by which intestinal congestion may contribute to the development of cardiac cachexia, is via lipopolysaccharide-induced production of pro-inflammatory cytokines [165]. The translocation of lipopolysaccharide into

the systemic circulation seems to be abetted during edematous decompensation [166], possibly as a consequence of acute intestinal congestion.
Extending this finding, we suggest that chronic intestinal congestion, such as the one found in cardiac cachexia in HF-patients, may cause a persistent lipopolysaccharide translocation even in stable patients. Our finding of higher serum levels of hsCRP in cachectic patients, which correlated with larger BWT, supports this view.

As mentioned above cachexia as comorbidity of HF is associated with anorexia. We assessed in a further study the determinants of anorexia in patients with HF and aimed to further elucidate the association between anorexia, functional capacity, and outcomes in affected patients. **"Anorexia, functional capacity, and clinical outcome in patients with chronic heart failure"**. We found that more than one-third of clinically stable ambulatory patients with HF reported any anorexia. Using multivariable regression analysis, we identified an increased level of inflammation, loop diuretic use, and the presence of cachexia as major determinants of anorexia in patients with HF. Our findings demonstrate that patients with anorexia are more likely to present with impaired functional capacity and higher all-cause mortality within 2 years. Moreover, cachexia showed additive effects of worsening functional capacity and poor outcomes in patients with HF and anorexia.
Previous studies have shown that anorexia is not an unavoidable consequence of chronic disease, and it often promotes its development through various mechanisms [167].
In our study, cachexia was more frequently detected in patients with anorexia (36.8%) compared with those without (18.2%), and cachexia remained an independent predictor of anorexia in our cohort. The complex clinical nature of HF-induced anorexia or HF-induced cachexia suggested a multifactorial etiology. In addition to cachexia, inflammation, medication side effects, depression, anxiety, sodium restricted diets, and chewing problem may also be found as cause of anorexia [168].
Anorexia–cachexia syndrome is often associated with the underlying disease process and related to both peripheral and central neurohormonal signals regulating both appetite and energy expenditure. Moreover, cachexia was related to intestinal congestion and can further worsen anorexia and impaired food intake in patients with HF [164].

An increase in circulating inflammatory cytokines including CRP, which is the most frequently measured inflammatory marker is one of the pathogenic mechanisms of anorexia and food intake in chronic disease [81]. One of the targets of inflammatory mediators is the central

nervous system, particularly on regulatory feeding centers in the hypothalamus sited in the ventral diencephalon. Recently, several studies explained the mechanisms by which inflammation reaches the hypothalamus and the neural substrates underlying inflammatory anorexia [169]. Additionally, pro-inflammatory cytokines persistently activate pro-opiomelanocortin neurons and inhibit neuropeptide Y neurons, and both are involved in the alteration of satiety and hunger signals (the clinical signs of cachexia and anorexia) [170].

Conventional HF-Therapy such as angiotensin converting enzyme inhibitors reduces the risk of weight loss [171]. One of the most important issues for managing anorexia is to maintain optimal HF treatment. On the other hand, several HF-treatments could contribute to inadequate food intake, especially overzealous diuretic use, which can cause anorexia in animal experiments [172].

For instance, the use of loop diuretics is associated with up-regulation of the activity of the renin–angiotensin–aldosterone system [173], and higher doses of diuretics are associated with poor outcomes in patients with HF. Angiotensin II, the primary contributor of renin–angiotensin–aldosterone system, causes anorexia and reduces food intake in rodents [174].

Our findings show declined functional capacity including SPPB score, distance of 6 MWT, and peak VO2 in patients with HF and anorexia. This is in line with previous research results [175, 176].

The frequency of anorexia symptoms is associated with the presence of sarcopenia in our patients. In addition, cachexia showed an additive effect in worsening functional capacity in patients with HF and anorexia. Therefore, it is crucial to detect the presence of anorexia and cachexia to reduce comorbidities and to preserve functional capacity in patients with HF.

Another important finding of our study is the association between anorexia and increased mortality. Older nursing home residents with anorexia had a higher risk of death for all-causes compared with non-anorexic participants [168]. In chronic renal failure, mortality risk in anorexic patients was 4 to 5 times that of those without anorexia [177].

However, there are few reports pointing out the association between anorexia and clinical outcome in patients with HF.

We will focus on the following page on a further relevant comorbidity of HF, namely anemia and iron deficiency and its relation to muscle function and exercise capacity.

ID may attribute to the explanation of exercise intolerance in patients with HFrEF [39]. However, no data were available about patients with HFpEF at the time of our publication. In

the article **"Iron deficiency in patients with heart failure with preserved ejection fraction and its association with reduced exercise capacity, muscle strength and quality of life"**, we described for the first time in a multicenter European study the prevalence of ID in patients with HFpEF (LVEF≥50%).

We found that about 60% of the whole cohort had ID. ID was more prevalent (87%) in patients with more severe diastolic dysfunction (E/e′ ≥ 15). Patients with ID performed worse during the 6MWT and had worse exercise time in the CPET.

Exercise intolerance in HFpEF patients might be related to anemia, insufficient oxygen supply or impaired oxygen use by the skeletal muscle during exercise [178, 179]. Iron plays a key role in oxygen uptake, transport, and storage, as well as oxidative metabolism in the skeletal muscle; furthermore, it is also involved in erythropoiesis [90]. It is known that absorption of iron in cases of inflammatory disorders is reduced due to intestinal edema and other factors [180, 181]. Moreover, iron can accumulate inside reticuloendothelial stores, which reduces the availability of iron for target tissues and stores despite adequate iron stores in the body, a phenomenon known as functional iron deficiency [182]. Furthermore, diastolic dysfunction has been shown to be associated with reduced cardiac energetic reserve [183]. Here, iron plays also an essential role in oxygen metabolism and cellular energetics. This is of special importance in the diastolic phase of the cardiac cycle including LV relaxation and filling due to the crucial role of sufficient cellular energetic supply through adenosine triphosphate (ATP) in the physiology of this phase. Therefore, ID may lead to an impairment of LV diastolic function and cardiac performance as well as reduced exercise capacity through impaired energetic balance and abnormal oxidative mitochondrial function [92]. As a result, the maintenance of normal iron metabolism and iron storage appears important for the maintenance of cardiac function and physiology [184].

The treatment of ID in patients with HF and an LVEF <45% and ID in the FAIR-HF trial showed an improvement in 6-MWT distance and QoL after 24 weeks [39]. In the CONFIRM-HF study, this therapy reduced the hospitalization rate after 52 weeks, a result confirmed in a recent meta-analysis [185]. The FAIR-HFpEF trial is enrolling currently patients with HFpEF and ID for the substitution of intravenous iron (NCT03074591). Just like in the FAIR-HF and the CONFIRM-HF trials, the primary outcome of this study is exercise capacity after intravenous iron administration in patients with HFpEF.

Other forgotten comorbidities of HF are psychological and mental disorders such as depression and anxiety, which are as mentioned earlier, common in both HFpEF and HFrEF [99, 100] and

have been proved to be independently associated with higher mortality and readmission rates [98, 101].

We investigated in the following paper **"Psychosocial factors, mental health, and coordination capacity in patients with HFpEF compared to HFrEF"** the prevalence of depression and anxiety in stable outpatients with HFpEF and HFrEF and its relation to muscle strength and coordination capacity. We found for the first time to our knowledge a profound reduction in QoL in patients with HFpEF assessed by mental health and vitality as estimated in the SF-36 questionnaire and increased anxiety and depression scores in the HADS questionnaire compared to those with HFrEF and non-HF controls. Both HFpEF and HFrEF showed reduced coordination capacities compared to non-HF controls. Elevated levels of inflammatory biomarkers were associated with reduced QoL and impaired coordination capacity. In a logistic regression the presence of at least borderline depression (≥8 points in the HADS-questionnaire) remained an independent factor for predicting reduced coordination capacity in the dynamic balance tests after adjusting for peak VO2, GDF-15, 10-meter walk test, physical component score of the SF-36 questionnaire, and peak torque of the right leg in extension.

Muscle strength and the balance between knee concentric and eccentric movements as well as the speed of developing peak torque are important factors to stabilize the gait and prevent against falls especially in elderly [186-190]. Furthermore, the muscular activation pattern during concentric and eccentric isokinetic movements seems to be different with a higher frequency of motor units during the eccentric muscle performance [191]. This emphasizes the importance of eccentric movements of the knee in creating higher strength and as a result of more gait balance. Our findings are supportive in this regard. We found that coordination capacity and peak torque of knee in eccentric extension and after adjusting to sex were significantly lower in patients with HFpEF and HFrEF than in non-HF controls. In addition, there was a correlation between muscle strength especially in eccentric movements of the knee and balance dynamic tests. This all makes patients with HF more susceptible to falls and to the following health-related, social, and economic consequences and emphasizes the importance of normal muscle function in stabilizing gait performance and improving coordination capacity, and QoL.

In conclusion, clinically stable outpatients with HFpEF have worse QoL and elevated prevalence of anxiety and depression compared to those with HFrEF and non-HF controls.

Depression was associated with reduced QoL and is an independent predictor for reduced coordination capacity.

Liver disorders as a comorbidity of HF is another example of an underestimated and rarely investigated comorbidity.
We aimed in our article **"Association of liver fibrosis risk scores with clinical outcomes in patients with heart failure with preserved ejection fraction"** to address these gaps in knowledge by describing the prevalence of liver fibrosis, as assessed using surrogate biomarkers, and the association of such biomarkers in predicting clinical outcomes in patients with HFpEF from the TOPCAT trial.
We found in a large contemporary HFpEF cohort with NAFLD risk factors including obesity and diabetes a high prevalence (ranging 8-38%) of risk for advanced liver fibrosis as measured by scoring systems. This finding underscores the importance of considering NAFLD, which may be masquerading as congestive hepatopathy, in HFpEF patients with mild-moderate elevations in AST/ALT.
Neither the NFS nor the FIB4 score correlated with the combined cardiovascular outcome measure after adjustment for potential confounders. However, a high FIB4 score remained significantly associated with hospitalization for HF, even after multivariable adjustment.
Further, unadjusted models did demonstrate significant associations, suggesting some overlap between liver disease and comorbidities regarding their effect on outcomes. For instance, obesity (included in NFS, and not in FIB4) appears to be a critical link in the pathologic process of myocardial remodelling in HF [113]. Additionally, the relationship between fibrosis score and outcomes may have been diluted by the heterogenous phenotypes in TOPCAT, some of which may not have been genuine HFpEF including a group of younger patients with milder symptoms and markers of lung disease, although some of these patients were enrolled in Eastern Europe and were excluded from this analysis [192]. Of note, liver disease was not captured as a predefined variable at baseline in TOPCAT, and aspartate transaminase (AST) and alanine transaminase (ALT) >3x upper level of normal was an exclusion criterion for the trial.

In conclusion, this study indicated that there may be a high prevalence of liver fibrosis in a large, contemporary HFpEF population along with a limited association between the risk for advanced liver fibrosis and clinical outcomes, mostly restricted to HF related events. Further research – namely, the inclusion of 'gold standard' liver disease instruments such as liver imaging and/or biopsy in HFpEF – is necessary to better understand the complex interplay

between chronic liver disease and HFpEF and determine whether there are any clear causal interactions.

The last presented comorbidity of HF in this thesis is SDB. As mentioned earlier, SDB is very common in patients with HF and patients with cardiac disease in general such as atrial fibrillation or severe valve disorders.
Our study" **Heart failure and sleep-disordered breathing: Susceptibility to muscle wasting and preclinical congestion"** was the first study that examined the differences in body composition in a cohort of patients with HF between those with and without SDB. We found significantly higher amounts of total body water (TBW), intra-cellular (ICW) and extra-cellular (ECW) in patients with SDB as a sign of preclinical congestion. Increased amounts of water were associated with an elevated AHI, reduced muscle strength, and exercise capacity as well as with an elevated basic metabolic rate. Altogether, patients with SDB showed worse cardiac function, reduced exercise capacity and muscle strength compared to those without SDB.

SDB remains underdiagnosed and often unrecognized due to its chronic and insidious incidence[193]. Patients may report non-specific symptoms such as excessive daytime somnolence, poor sleep quality, recurrent arrhythmias, nocturnal angina, and refractory HF symptoms [194]. However, such symptoms may be missing in patients with HF or strongly overlap with the symptoms deemed specific for the HF syndrome itself. The main two types of SDB are OSA, which is the most common form and presents with fewer symptoms, and the CSA, which is more often associated with more advanced HF and atrial fibrillation. Cheyne-Stokes respiration is typically seen in CSA. Both CSA and OSA are independent risk factors for ventricular arrhythmias, that requires cardioverter-defibrillator therapy [195].

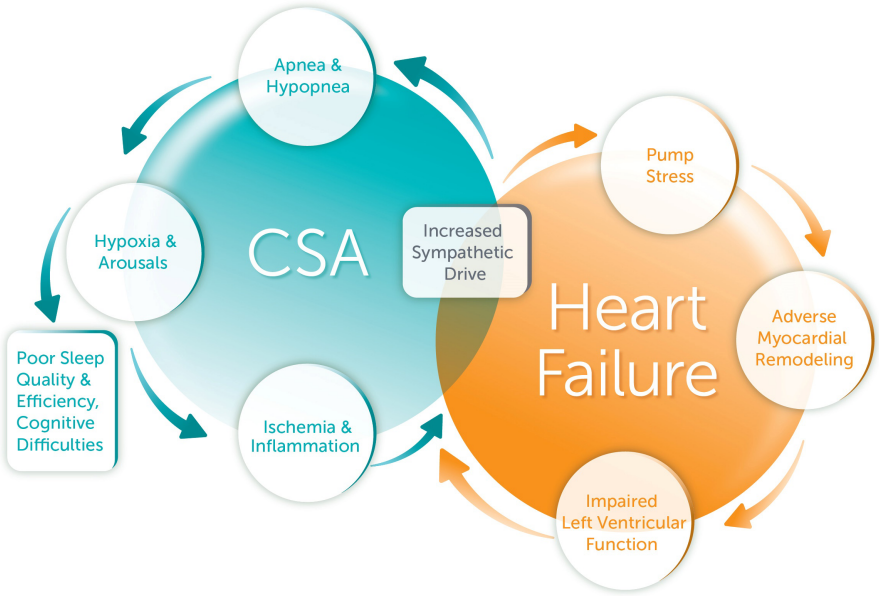
In the present study, we used ambulatory nocturnal screening devices and rescored these tests visually to classify patients into no SDB, SDB with CSA, OSA or mixed SDB accordingly. We found similar prevalence rates of SDB like those described in other studies [114, 115, 196]. About 67% of the whole cohort had any form of SDB. Overall, about 42% had OSA and 22% CSA[115].

It is known that the combination of HF and SDB leads to higher sympathetic nervous activity during wakefulness[197]. This will lead in turn to increase the metabolic rate and the catecholamine surge and may accelerate the progression of HF[198]. Another adverse effect of

increased sympathetic activation is provoking the catabolic cascade and the protein degradation in skeletal muscle that could eventually lead to skeletal muscle wasting (sarcopenia) and reduced muscle function [199]. We found that 17 of 111 patients presented with skeletal muscle wasting. Of these 11 (65%) patients had SDB vs. 6 patients (35%) without SDB. This was statistically not significant, which is likely a consequence of the small sample volume. Our previous results in both patients with HFpEF and HFrEF signified the clinical importance of muscle wasting showing its association with reduced exercise capacity, muscle strength and QoL [59, 64]. The prevalence of muscle wasting and its clinical significance in patients with HF and SDB need to be confirmed in further and larger studies.

The pathophysiology and the relationship between HF and SDB, especially CSA, have been recently suggested in one of my previous publications [115]. It is known that patients with HF tend to hyperventilate [200]. This could be related to interstitial pulmonary congestion as a result of the fluid redistribution in the supine position and could also lead to the activation of pulmonary stretch receptors and to the stimulation of ventilation yielding relative hypocapnia [201]. Each episode of apnea and arousal results in hypoxia, norepinephrine release, and wide oscillations in carbon dioxide [202]. Hypoxia results in cardiac ischemia and elevated norepinephrine causes atrial and ventricular arrhythmias [203] and activates the renin–angiotensin system resulting in sodium retention and neurohormonal activation [198]. Neurohormonal activation and ischemia further activate central and peripheral chemoreceptors and baroreceptors, both of which destabilize breathing and trigger CSA [204]. This causes additional pump stress leading to adverse myocardial remodeling and downward progression of HF [205] **(Figure 15)**.

By showing increased amounts of water in patients with SDB and HF, our results underscore an important preclinical finding. Indeed, there was no clinical difference regarding



Bekfani T, Abraham WT. Current and future developments in the field of central sleep apnoea. Europace. 2016 Aug;18(8):1123-34.

Figure 15: Pathophysiology and the relationship between HF, CSA and arrhythmias.

the signs of congestive HF such as elevated jugular vein pressure or ankle edema between patients with and without SDB. Since therapies for OSA [206] and CSA [207] are available and because of the high prevalence of this HF-comorbidity, an active screening program for SDB in patients with HF is highly recommended [115]. Such screening should also include BIA assessment or other similar techniques to detect preclinical congestion in patients with HF especially in those who additionally have further comorbidities such as SDB.

Previous studies showed the lack of an association between central fat mass effect and OSA in elderly patients [208]. This finding was confirmed by our results. Other studies using BIA demonstrated a relationship between the severity of neck fluid volume of water and the severity of SDB. This could be explained by the physiology and pathophysiology of both HF and SDB [201, 209]. Overnight rostral fluid displacement from the legs could play a role in the development of OSA independent of body weight [210]. Therefore, screening for preclinical congestion in HF should be recommended, particularly in those with SDB or otherwise more advanced disease. Further non-invasive and invasive monitoring in patients with HF and SDB could be necessary. The TIM-HF2 suggested recently that a structured remote patient management intervention in patients with HF could reduce the percentage of days lost due to unplanned cardiovascular hospital admissions and all-cause mortality [211]. Invasive procedures like implantation of CardioMems^{TM} devices seem to be promising and could be

necessary for achieving a successful monitoring in this group of patients. In a single study, CardioMems^{TM} implantation resulted in an 80.4% reduction in HF admissions and a 69% reduction in all-cause admissions [212].

8. CONCLUSIONS AND FUTURE PERSPECTIVES

HF is a major public health issue with steadily increasing incidence and prevalence. Hospitalization and mortality due to HF remains high in spite of advancements in the management of HF [213, 214]. In some epidemiological analyses patients with HF showed 5 or more comorbidities. The risk of hospitalization is associated strongly with the number of comorbidities. Non-cardiovascular comorbidities are responsible for more than half of the hospitalizations and often lead to worse outcomes in patients with HF especially in those with HFpEF. Therefore, screening for comorbidities systematically in patients with HF is very important and could influence the outcome and the prognosis in patients with HF.

While advances in medical and device therapies have improved morbidity and mortality in patients with HFrEF, no benefits have been demonstrated in patients with HFpEF except for the recently published trial (EMPEROR Preserved trial) [215-219]. A further proven therapy to improve exercise capacity, dyspnea, and QoL in patients with HFpEF is exercise training [220]. The main symptoms in patients with HF are dyspnea and exercise intolerance. Some studies showed that exercise intolerance can remain for several months after heart transplantation and achieving improvement of cardiac output [221]. Furthermore, exercise training improves exercise tolerance in patients with HF independent of improving cardiac function [77]. Both examples show that skeletal muscle function may play a central role in explaining the limited exercise capacity in patients with HF [222].

I investigated in this cumulative thesis comorbidities of HF with the focus on wasting disorders, especially those related to skeletal muscle. The performed studies varied from basic science research to clinical research. The focus was not only on metabolic, mitochondrial and structure changes on molecular level, but also on clinically related questions such as exercise capacity, QoL and mortality related to some comorbidities of HF. **Figure 16** represents a graphical summary of the investigated comorbidities of heart failure in this Habilitation thesis.

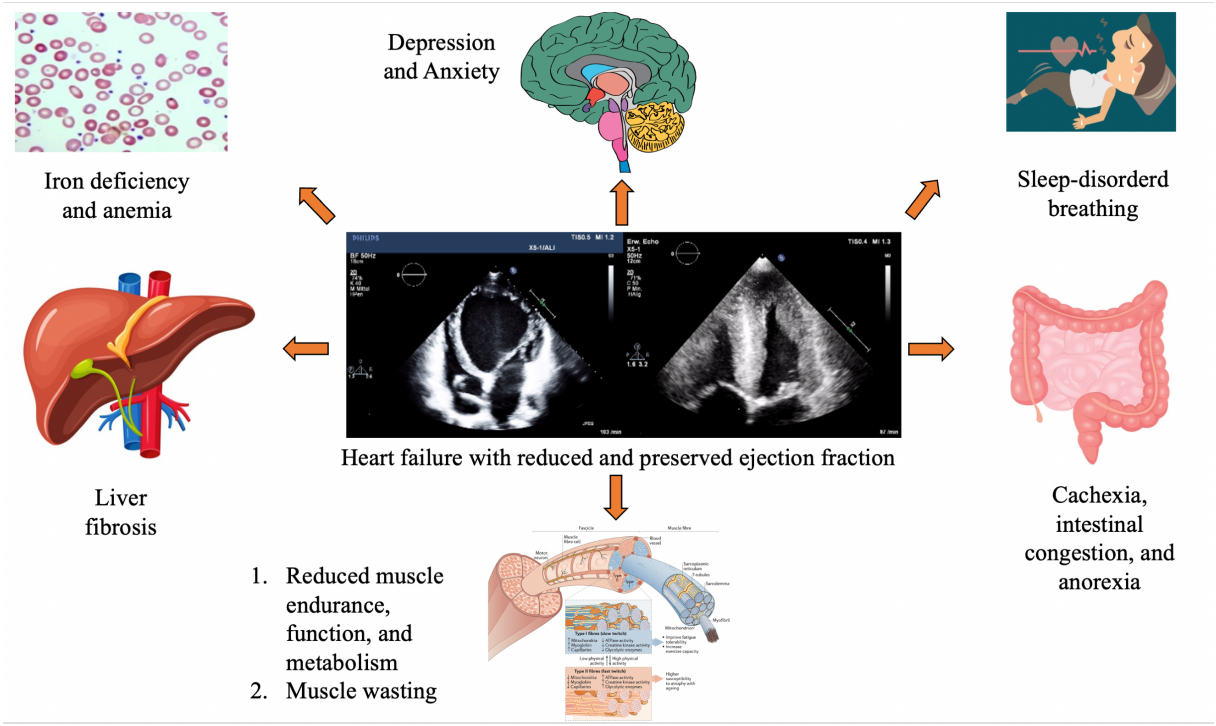


Figure 16: Graphical summary of the investigated comorbidities of heart failure in this Habilitation thesis.

Since for the treatment of patients with HFpEF limited evidence exists in spite of the high prevalence and incidence of this syndrome, understanding changes taking place on the level of skeletal muscle becomes of special importance in this group of patients. Our findings showed increased levels of atrophy genes and proteins as well as reduced mitochondrial size in skeletal muscle in stable outpatients with HFpEF compared with HFrEF and HC. Additionally, the link between inflammation and reduced muscle endurance should support future research in switching the focus at least partially to peripheral factors aiming to develop new therapies for patients with HFpEF to improve exercise capacity and dyspnea, which are the main manifestations in HFpEF.

A further supporting study to focus on skeletal muscle function and skeletal muscle wasting is the increased mortality in patients with skeletal muscle wasting and HF as shown above.

Detecting RME could be challenging in routine clinical work, thus biomarkers could be very important in early detecting RME. We found in this regard that both Kyn and LAEF (LAS) are promising biomarkers in detecting RME.

Further manifestations of wasting disorders such as cachexia and anorexia have been proven to be very prevalent and highly clinically relevant in patients with HF as shown above.

ID is a further example of common comorbidities especially in patients with HFpEF, that have not received enough interest yet. Our study was the first one to show the prevalence of ID in HFpEF patients and its relationship to exercise capacity and QoL. Studies are currently running to evaluate the effect of iron supplementation in patients with HFpEF on exercise capacity.

SDB, anxiety, depression, and liver fibrosis are additional example of under-investigated comorbidities of HF. As shown in the above-discussed studies these are very relevant and common comorbidities and are associated with reduced exercise capacity and QoL.

Therefore, increasing the awareness on HF-comorbidities and integrating related screening tests as part of the routine evaluations of patients with HF is essential. This will not only have impact on morbidity and mortality, exercise capacity and QoL of patients with HF but will also lead to reduce the health care costs arising from frequent hospitalizations and readmissions as a result of HF decompensations.

9. REFERNCES

1. McDonagh, T.A., et al., *2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure.* Eur Heart J, 2021. **42**(36): p. 3599-3726.
2. von Haehling, S., et al., *Diabetes mellitus, cachexia and obesity in heart failure: rationale and design of the Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF).* J Cachexia Sarcopenia Muscle, 2010. **1**(2): p. 187-194.
3. Nagueh, S.F., et al., *Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging.* J Am Soc Echocardiogr, 2016. **29**(4): p. 277-314.
4. Ponikowski, P., et al., *2016 ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure.* Rev Esp Cardiol (Engl Ed), 2016. **69**(12): p. 1167.
5. Ambrosy, A.P., et al., *The global health and economic burden of hospitalizations for heart failure: lessons learned from hospitalized heart failure registries.* J Am Coll Cardiol, 2014. **63**(12): p. 1123-1133.
6. Ambrosy, A.P., et al., *Global perspectives in hospitalized heart failure: regional and ethnic variation in patient characteristics, management, and outcomes.* Curr Heart Fail Rep, 2014. **11**(4): p. 416-27.
7. Zarrinkoub, R., et al., *The epidemiology of heart failure, based on data for 2.1 million inhabitants in Sweden.* Eur J Heart Fail, 2013. **15**(9): p. 995-1002.
8. Conrad, N., et al., *Temporal trends and patterns in heart failure incidence: a population-based study of 4 million individuals.* Lancet, 2018. **391**(10120): p. 572-580.
9. Pan, A., *The real-world evidence of heart failure co-morbidities.* Eur J Heart Fail, 2017. **19**(3): p. 434.
10. Group, C.T.S., *Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS).* N Engl J Med, 1987. **316**(23): p. 1429-35.
11. Hjalmarson, A., et al., *Effects of controlled-release metoprolol on total mortality, hospitalizations, and well-being in patients with heart failure: the Metoprolol CR/XL Randomized Intervention Trial in congestive heart failure (MERIT-HF). MERIT-HF Study Group.* JAMA, 2000. **283**(10): p. 1295-302.
12. Packer, M., et al., *Effect of carvedilol on the morbidity of patients with severe chronic heart failure: results of the carvedilol prospective randomized cumulative survival (COPERNICUS) study.* Circulation, 2002. **106**(17): p. 2194-9.

13. *The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial.* Lancet, 1999. **353**(9146): p. 9-13.
14. McMurray, J.J., et al., *Angiotensin-neprilysin inhibition versus enalapril in heart failure.* N Engl J Med, 2014. **371**(11): p. 993-1004.
15. Moss, A.J., et al., *Reduction in inappropriate therapy and mortality through ICD programming.* N Engl J Med, 2012. **367**(24): p. 2275-83.
16. Heidenreich, P.A., et al., *Forecasting the impact of heart failure in the United States: a policy statement from the American Heart Association.* Circ Heart Fail, 2013. **6**(3): p. 606-19.
17. Dunlay, S.M. and V.L. Roger, *Understanding the epidemic of heart failure: past, present, and future.* Curr Heart Fail Rep, 2014. **11**(4): p. 404-15.
18. Epstein, A.M., A.K. Jha, and E.J. Orav, *The relationship between hospital admission rates and rehospitalizations.* N Engl J Med, 2011. **365**(24): p. 2287-95.
19. von Haehling, S., *Co-morbidities in heart failure beginning to sprout-and no end in sight?* Eur J Heart Fail, 2017. **19**(12): p. 1566-1568.
20. Chun, S., et al., *Lifetime analysis of hospitalizations and survival of patients newly admitted with heart failure.* Circ Heart Fail, 2012. **5**(4): p. 414-21.
21. van Oeffelen, A.A., et al., *Prognosis after a first hospitalisation for acute myocardial infarction and congestive heart failure by country of birth.* Heart, 2014. **100**(18): p. 1436-43.
22. Ryan, A.M., B.K. Nallamothu, and J.B. Dimick, *Medicare's public reporting initiative on hospital quality had modest or no impact on mortality from three key conditions.* Health Aff (Millwood), 2012. **31**(3): p. 585-92.
23. Maggioni, A.P., et al., *The real-world evidence of heart failure: findings from 41 413 patients of the ARNO database.* Eur J Heart Fail, 2016. **18**(4): p. 402-10.
24. Soundarraj, D., et al., *Containing the Cost of Heart Failure Management: A Focus on Reducing Readmissions.* Heart Fail Clin, 2017. **13**(1): p. 21-28.
25. Benjamin, E.J., et al., *Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association.* Circulation, 2018. **137**(12): p. e67-e492.
26. Barasa, A., et al., *Heart failure in young adults: 20-year trends in hospitalization, aetiology, and case fatality in Sweden.* Eur Heart J, 2014. **35**(1): p. 25-32.
27. Henkel, D.M., et al., *Death in heart failure: a community perspective.* Circ Heart Fail, 2008. **1**(2): p. 91-7.

28. Dunlay, S.M., et al., *Risk factors for heart failure: a population-based case-control study.* Am J Med, 2009. **122**(11): p. 1023-8.
29. Redfield, M.M., et al., *Age- and gender-related ventricular-vascular stiffening: a community-based study.* Circulation, 2005. **112**(15): p. 2254-62.
30. Seferovic, P.M., et al., *Heart failure in cardiomyopathies: a position paper from the Heart Failure Association of the European Society of Cardiology.* Eur J Heart Fail, 2019. **21**(5): p. 553-576.
31. Wong, C.Y., et al., *Trends in comorbidity, disability, and polypharmacy in heart failure.* Am J Med, 2011. **124**(2): p. 136-43.
32. Braunstein, J.B., et al., *Noncardiac comorbidity increases preventable hospitalizations and mortality among Medicare beneficiaries with chronic heart failure.* J Am Coll Cardiol, 2003. **42**(7): p. 1226-33.
33. Hawkins, N.M., S. Virani, and C. Ceconi, *Heart failure and chronic obstructive pulmonary disease: the challenges facing physicians and health services.* Eur Heart J, 2013. **34**(36): p. 2795-803.
34. Enjuanes, C., et al., *Iron deficiency and health-related quality of life in chronic heart failure: results from a multicenter European study.* Int J Cardiol, 2014. **174**(2): p. 268-75.
35. Desai, A.S., *Hyperkalemia in patients with heart failure: incidence, prevalence, and management.* Curr Heart Fail Rep, 2009. **6**(4): p. 272-80.
36. Ronco, C., et al., *Cardiorenal syndrome.* J Am Coll Cardiol, 2008. **52**(19): p. 1527-39.
37. Damman, K., et al., *Renal impairment, worsening renal function, and outcome in patients with heart failure: an updated meta-analysis.* Eur Heart J, 2014. **35**(7): p. 455-69.
38. Eschenhagen, T., et al., *Cardiovascular side effects of cancer therapies: a position statement from the Heart Failure Association of the European Society of Cardiology.* Eur J Heart Fail, 2011. **13**(1): p. 1-10.
39. Anker, S.D., et al., *Ferric carboxymaltose in patients with heart failure and iron deficiency.* N Engl J Med, 2009. **361**(25): p. 2436-48.
40. Mentz, R.J., et al., *Noncardiac comorbidities in heart failure with reduced versus preserved ejection fraction.* J Am Coll Cardiol, 2014. **64**(21): p. 2281-93.
41. van Deursen, V.M., et al., *Co-morbidities in patients with heart failure: an analysis of the European Heart Failure Pilot Survey.* Eur J Heart Fail, 2014. **16**(1): p. 103-11.

42. Sarma, S., et al., *Association between diabetes mellitus and post-discharge outcomes in patients hospitalized with heart failure: findings from the EVEREST trial.* Eur J Heart Fail, 2013. **15**(2): p. 194-202.
43. Parissis, J.T., et al., *Acute heart failure in patients with diabetes mellitus: clinical characteristics and predictors of in-hospital mortality.* Int J Cardiol, 2012. **157**(1): p. 108-13.
44. Ceia, F., et al., *Prevalence of chronic heart failure in Southwestern Europe: the EPICA study.* Eur J Heart Fail, 2002. **4**(4): p. 531-9.
45. Greenberg, B.H., et al., *Influence of diabetes on characteristics and outcomes in patients hospitalized with heart failure: a report from the Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure (OPTIMIZE-HF).* Am Heart J, 2007. **154**(2): p. 277 e1-8.
46. Lam, C.S., *Diabetic cardiomyopathy: An expression of stage B heart failure with preserved ejection fraction.* Diab Vasc Dis Res, 2015. **12**(4): p. 234-8.
47. Seferovic, P.M. and W.J. Paulus, *Clinical diabetic cardiomyopathy: a two-faced disease with restrictive and dilated phenotypes.* Eur Heart J, 2015. **36**(27): p. 1718-27, 1727a-1727c.
48. Tenenbaum, A., et al., *Functional class in patients with heart failure is associated with the development of diabetes.* Am J Med, 2003. **114**(4): p. 271-5.
49. Levy, D., et al., *The progression from hypertension to congestive heart failure.* JAMA, 1996. **275**(20): p. 1557-62.
50. Chao, T.F., et al., *Lifetime Risks, Projected Numbers, and Adverse Outcomes in Asian Patients With Atrial Fibrillation: A Report From the Taiwan Nationwide AF Cohort Study.* Chest, 2018. **153**(2): p. 453-466.
51. Lamassa, M., et al., *Characteristics, outcome, and care of stroke associated with atrial fibrillation in Europe: data from a multicenter multinational hospital-based registry (The European Community Stroke Project).* Stroke, 2001. **32**(2): p. 392-8.
52. Ferreira, J.P. and M. Santos, *Heart failure and atrial fibrillation: from basic science to clinical practice.* Int J Mol Sci, 2015. **16**(2): p. 3133-47.
53. Santhanakrishnan, R., et al., *Atrial Fibrillation Begets Heart Failure and Vice Versa: Temporal Associations and Differences in Preserved Versus Reduced Ejection Fraction.* Circulation, 2016. **133**(5): p. 484-92.
54. Ling, L.H., et al., *Comorbidity of atrial fibrillation and heart failure.* Nat Rev Cardiol, 2016. **13**(3): p. 131-47.

55. Guder, G., et al., *Chronic obstructive pulmonary disease in heart failure: accurate diagnosis and treatment.* Eur J Heart Fail, 2014. **16**(12): p. 1273-82.
56. Brenner, S., et al., *Airway obstruction in systolic heart failure--COPD or congestion?* Int J Cardiol, 2013. **168**(3): p. 1910-6.
57. Rosenberg, I.H., *Sarcopenia: origins and clinical relevance.* J Nutr, 1997. **127**(5 Suppl): p. 990S-991S.
58. Morley, J.E., S.D. Anker, and S. von Haehling, *Prevalence, incidence, and clinical impact of sarcopenia: facts, numbers, and epidemiology-update 2014.* J Cachexia Sarcopenia Muscle, 2014. **5**(4): p. 253-9.
59. Bekfani, T., et al., *Sarcopenia in patients with heart failure with preserved ejection fraction: Impact on muscle strength, exercise capacity and quality of life.* Int J Cardiol, 2016. **222**: p. 41-46.
60. Heymsfield, S.B., et al., *Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry.* Am J Clin Nutr, 1990. **52**(2): p. 214-8.
61. Morley, J.E., et al., *Sarcopenia with limited mobility: an international consensus.* J Am Med Dir Assoc, 2011. **12**(6): p. 403-9.
62. Baumgartner, R.N., et al., *Epidemiology of sarcopenia among the elderly in New Mexico.* Am J Epidemiol, 1998. **147**(8): p. 755-63.
63. Anker, S.D., et al., *Muscle wasting disease: a proposal for a new disease classification.* J Cachexia Sarcopenia Muscle, 2014. **5**(1): p. 1-3.
64. Fulster, S., et al., *Muscle wasting in patients with chronic heart failure: results from the studies investigating co-morbidities aggravating heart failure (SICA-HF).* Eur Heart J, 2013. **34**(7): p. 512-9.
65. Bekfani, T., et al., *Skeletal Muscle Function, Structure, and Metabolism in Patients With Heart Failure With Reduced Ejection Fraction and Heart Failure With Preserved Ejection Fraction.* Circ Heart Fail, 2020. **13**(12): p. e007198.
66. Marcinkiewicz-Siemion, M., et al., *LC-MS-based serum fingerprinting reveals significant dysregulation of phospholipids in chronic heart failure.* J Pharm Biomed Anal, 2018. **154**: p. 354-363.
67. Maeder, M.T., et al., *Hemodynamic basis of exercise limitation in patients with heart failure and normal ejection fraction.* J Am Coll Cardiol, 2010. **56**(11): p. 855-63.
68. Kitzman, D.W., et al., *Exercise intolerance in patients with heart failure and preserved left ventricular systolic function: failure of the Frank-Starling mechanism.* J Am Coll Cardiol, 1991. **17**(5): p. 1065-72.

69. Morris, D.A., et al., *Normal values and clinical relevance of left atrial myocardial function analysed by speckle-tracking echocardiography: multicentre study.* Eur Heart J Cardiovasc Imaging, 2015. **16**(4): p. 364-72.
70. Morris, D.A., et al., *Potential Usefulness and Clinical Relevance of Adding Left Atrial Strain to Left Atrial Volume Index in the Detection of Left Ventricular Diastolic Dysfunction.* JACC Cardiovasc Imaging, 2018. **11**(10): p. 1405-1415.
71. Cameli, M., et al., *Correlation of Left Atrial Strain and Doppler Measurements with Invasive Measurement of Left Ventricular End-Diastolic Pressure in Patients Stratified for Different Values of Ejection Fraction.* Echocardiography, 2016. **33**(3): p. 398-405.
72. von Roeder, M., et al., *Influence of Left Atrial Function on Exercise Capacity and Left Ventricular Function in Patients With Heart Failure and Preserved Ejection Fraction.* Circ Cardiovasc Imaging, 2017. **10**(4).
73. D'Andrea, A., et al., *Association between left atrial myocardial function and exercise capacity in patients with either idiopathic or ischemic dilated cardiomyopathy: a two-dimensional speckle strain study.* Int J Cardiol, 2009. **132**(3): p. 354-63.
74. Leite, L., et al., *Left atrial mechanics strongly predict functional capacity assessed by cardiopulmonary exercise testing in subjects without structural heart disease.* Int J Cardiovasc Imaging, 2017. **33**(5): p. 635-642.
75. Chien, C.Y., et al., *Atrial deformation correlated with functional capacity in mitral stenosis patients.* Echocardiography, 2018. **35**(2): p. 190-195.
76. Vukomanovic, V., et al., *Is there association between left atrial function and functional capacity in patients with uncomplicated type 2 diabetes?* Int J Cardiovasc Imaging, 2020. **36**(1): p. 15-22.
77. Haykowsky, M.J., et al., *Effect of endurance training on the determinants of peak exercise oxygen consumption in elderly patients with stable compensated heart failure and preserved ejection fraction.* J Am Coll Cardiol, 2012. **60**(2): p. 120-8.
78. Anker, S.D., et al., *Wasting as independent risk factor for mortality in chronic heart failure.* Lancet, 1997. **349**(9058): p. 1050-3.
79. Anker, S.D., et al., *Hormonal changes and catabolic/anabolic imbalance in chronic heart failure and their importance for cardiac cachexia.* Circulation, 1997. **96**(2): p. 526-34.
80. Arutyunov, G.P., et al., *Collagen accumulation and dysfunctional mucosal barrier of the small intestine in patients with chronic heart failure.* Int J Cardiol, 2008. **125**(2): p. 240-5.

81. Braun, T.P. and D.L. Marks, *Pathophysiology and treatment of inflammatory anorexia in chronic disease.* J Cachexia Sarcopenia Muscle, 2010. **1**(2): p. 135-145.
82. Valentova, M., et al., *Cardiac cachexia is associated with right ventricular failure and liver dysfunction.* Int J Cardiol, 2013. **169**(3): p. 219-24.
83. Evans, W.J., et al., *Cachexia: a new definition.* Clin Nutr, 2008. **27**(6): p. 793-9.
84. Invernizzi, M., et al., *Possible synergism of physical exercise and ghrelin-agonists in patients with cachexia associated with chronic heart failure.* Aging Clin Exp Res, 2014. **26**(4): p. 341-51.
85. Landi, F., A. Laviano, and A.J. Cruz-Jentoft, *The anorexia of aging: is it a geriatric syndrome?* J Am Med Dir Assoc, 2010. **11**(3): p. 153-6.
86. Tarricone, R., et al., *Impact of cancer anorexia-cachexia syndrome on health-related quality of life and resource utilisation: A systematic review.* Crit Rev Oncol Hematol, 2016. **99**: p. 49-62.
87. Morley, J.E., *Anorexia, sarcopenia, and aging.* Nutrition, 2001. **17**(7-8): p. 660-3.
88. Blauwhoff-Buskermolen, S., et al., *The assessment of anorexia in patients with cancer: cut-off values for the FAACT-A/CS and the VAS for appetite.* Support Care Cancer, 2016. **24**(2): p. 661-666.
89. von Haehling, S., et al., *Anaemia among patients with heart failure and preserved or reduced ejection fraction: results from the SENIORS study.* Eur J Heart Fail, 2011. **13**(6): p. 656-63.
90. Dunn, L.L., Y. Suryo Rahmanto, and D.R. Richardson, *Iron uptake and metabolism in the new millennium.* Trends Cell Biol, 2007. **17**(2): p. 93-100.
91. von Haehling, S., et al., *Iron deficiency and cardiovascular disease.* Nat Rev Cardiol, 2015. **12**(11): p. 659-69.
92. Haas, J.D. and T.t. Brownlie, *Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship.* J Nutr, 2001. **131**(2S-2): p. 676S-688S; discussion 688S-690S.
93. Lewis, G.D., et al., *Effect of Oral Iron Repletion on Exercise Capacity in Patients With Heart Failure With Reduced Ejection Fraction and Iron Deficiency: The IRONOUT HF Randomized Clinical Trial.* JAMA, 2017. **317**(19): p. 1958-1966.
94. Klip, I.T., et al., *Iron deficiency in chronic heart failure: an international pooled analysis.* Am Heart J, 2013. **165**(4): p. 575-582 e3.
95. Jankowska, E.A., et al., *Iron deficiency: an ominous sign in patients with systolic chronic heart failure.* Eur Heart J, 2010. **31**(15): p. 1872-80.

96. Edelmann, F., et al., *Impaired physical quality of life in patients with diastolic dysfunction associates more strongly with neurohumoral activation than with echocardiographic parameters: quality of life in diastolic dysfunction.* Am Heart J, 2011. **161**(4): p. 797-804.
97. Gary, R.A., et al., *Home-based exercise improves functional performance and quality of life in women with diastolic heart failure.* Heart Lung, 2004. **33**(4): p. 210-8.
98. Nolte, K., et al., *Effects of exercise training on different quality of life dimensions in heart failure with preserved ejection fraction: the Ex-DHF-P trial.* Eur J Prev Cardiol, 2015. **22**(5): p. 582-93.
99. Easton, K., et al., *Prevalence and Measurement of Anxiety in Samples of Patients With Heart Failure: Meta-analysis.* J Cardiovasc Nurs, 2016. **31**(4): p. 367-79.
100. Rutledge, T., et al., *Depression in heart failure a meta-analytic review of prevalence, intervention effects, and associations with clinical outcomes.* J Am Coll Cardiol, 2006. **48**(8): p. 1527-37.
101. Lin, T.K., et al., *Prognostic Value of Anxiety Between Heart Failure With Reduced Ejection Fraction and Heart Failure With Preserved Ejection Fraction.* J Am Heart Assoc, 2019. **8**(12): p. e010739.
102. Angermann, C.E., et al., *Somatic correlates of comorbid major depression in patients with systolic heart failure.* Int J Cardiol, 2011. **147**(1): p. 66-73.
103. Agudelo, L.Z., et al., *Skeletal muscle PGC-1alpha1 modulates kynurenine metabolism and mediates resilience to stress-induced depression.* Cell, 2014. **159**(1): p. 33-45.
104. Reeves, G.R., et al., *Comparison of Frequency of Frailty and Severely Impaired Physical Function in Patients >/=60 Years Hospitalized With Acute Decompensated Heart Failure Versus Chronic Stable Heart Failure With Reduced and Preserved Left Ventricular Ejection Fraction.* Am J Cardiol, 2016. **117**(12): p. 1953-8.
105. Unalp-Arida, A. and C.E. Ruhl, *Liver fibrosis scores predict liver disease mortality in the United States population.* Hepatology, 2017. **66**(1): p. 84-95.
106. Paik, J.M., et al., *Mortality Related to Nonalcoholic Fatty Liver Disease Is Increasing in the United States.* Hepatol Commun, 2019. **3**(11): p. 1459-1471.
107. Konerman MA, M.J., Hummel SL, Konerman MC. , *Prevalence of and Characteristics Associated with Non-Alcoholic Fatty Liver Disease Among Patients with Heart Failure with Preserved Ejection Fraction.* J Card Fail 2018. **24:S5**(Available from: https://doi.org/10.1016/j.cardfail.2018.07.147).

108. Yoshihisa, A., et al., *Liver fibrosis score predicts mortality in heart failure patients with preserved ejection fraction.* ESC Heart Fail, 2018. **5**(2): p. 262-270.
109. Wijarnpreecha, K., et al., *Association between diastolic cardiac dysfunction and nonalcoholic fatty liver disease: A systematic review and meta-analysis.* Dig Liver Dis, 2018. **50**(11): p. 1166-1175.
110. VanWagner, L.B., et al., *Association of nonalcoholic fatty liver disease with subclinical myocardial remodeling and dysfunction: A population-based study.* Hepatology, 2015. **62**(3): p. 773-83.
111. Pacifico, L., et al., *Left ventricular dysfunction in obese children and adolescents with nonalcoholic fatty liver disease.* Hepatology, 2014. **59**(2): p. 461-70.
112. So-Armah, K.A., et al., *FIB-4 stage of liver fibrosis is associated with incident heart failure with preserved, but not reduced, ejection fraction among people with and without HIV or hepatitis C.* Prog Cardiovasc Dis, 2020. **63**(2): p. 184-191.
113. VanWagner, L.B., et al., *Longitudinal Association of Non-Alcoholic Fatty Liver Disease With Changes in Myocardial Structure and Function: The CARDIA Study.* J Am Heart Assoc, 2020. **9**(4): p. e014279.
114. Oldenburg, O., et al., *Sleep-disordered breathing in patients with symptomatic heart failure: a contemporary study of prevalence in and characteristics of 700 patients.* Eur J Heart Fail, 2007. **9**(3): p. 251-7.
115. Bekfani, T. and W.T. Abraham, *Current and future developments in the field of central sleep apnoea.* Europace, 2016. **18**(8): p. 1123-34.
116. Bezerra, P.C., et al., *The use of dual-energy X-ray absorptiometry in the evaluation of obesity in women with obstructive sleep apnea-hypopnea syndrome.* Eur Arch Otorhinolaryngol, 2013. **270**(4): p. 1539-45.
117. Segal, K.R., et al., *Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study.* Am J Clin Nutr, 1988. **47**(1): p. 7-14.
118. Pedroso, F.E., et al., *Inflammation, organomegaly, and muscle wasting despite hyperphagia in a mouse model of burn cachexia.* J Cachexia Sarcopenia Muscle, 2012. **3**(3): p. 199-211.
119. von Haehling, S., et al., *Muscle wasting and cachexia in heart failure: mechanisms and therapies.* Nat Rev Cardiol, 2017. **14**(6): p. 323-341.
120. Chau, V., et al., *A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein.* Science, 1989. **243**(4898): p. 1576-83.

121. Passmore, L.A. and D. Barford, *Getting into position: the catalytic mechanisms of protein ubiquitylation.* Biochem J, 2004. **379**(Pt 3): p. 513-25.
122. Bodine, S.C., et al., *Identification of ubiquitin ligases required for skeletal muscle atrophy.* Science, 2001. **294**(5547): p. 1704-8.
123. Cai, D., et al., *IKKbeta/NF-kappaB activation causes severe muscle wasting in mice.* Cell, 2004. **119**(2): p. 285-98.
124. Sishi, B.J. and A.M. Engelbrecht, *Tumor necrosis factor alpha (TNF-alpha) inactivates the PI3-kinase/PKB pathway and induces atrophy and apoptosis in L6 myotubes.* Cytokine, 2011. **54**(2): p. 173-84.
125. Stahrenberg, R., et al., *The novel biomarker growth differentiation factor 15 in heart failure with normal ejection fraction.* Eur J Heart Fail, 2010. **12**(12): p. 1309-16.
126. Scarpulla, R.C., *Transcriptional paradigms in mammalian mitochondrial biogenesis and function.* Physiol Rev, 2008. **88**(2): p. 611-38.
127. Esposito, F., et al., *Limited maximal exercise capacity in patients with chronic heart failure: partitioning the contributors.* J Am Coll Cardiol, 2010. **55**(18): p. 1945-54.
128. Liesa, M., M. Palacin, and A. Zorzano, *Mitochondrial dynamics in mammalian health and disease.* Physiol Rev, 2009. **89**(3): p. 799-845.
129. Chen, H., et al., *Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development.* J Cell Biol, 2003. **160**(2): p. 189-200.
130. Lopaschuk, G.D., et al., *Myocardial fatty acid metabolism in health and disease.* Physiol Rev, 2010. **90**(1): p. 207-58.
131. Zhang, J., et al., *Human skeletal muscle PPARalpha expression correlates with fat metabolism gene expression but not BMI or insulin sensitivity.* Am J Physiol Endocrinol Metab, 2004. **286**(2): p. E168-75.
132. von Haehling, S., *The wasting continuum in heart failure: from sarcopenia to cachexia.* Proc Nutr Soc, 2015. **74**(4): p. 367-77.
133. Dos Santos, M.R., et al., *Sarcopenia and Endothelial Function in Patients With Chronic Heart Failure: Results From the Studies Investigating Comorbidities Aggravating Heart Failure (SICA-HF).* J Am Med Dir Assoc, 2017. **18**(3): p. 240-245.
134. Piepoli, M.F., et al., *Reduced peripheral skeletal muscle mass and abnormal reflex physiology in chronic heart failure.* Circulation, 2006. **114**(2): p. 126-34.
135. Saitoh, M., et al., *Anorexia, functional capacity, and clinical outcome in patients with chronic heart failure: results from the Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF).* ESC Heart Fail, 2017. **4**(4): p. 448-457.

136. Dziegala, M., et al., *Iron deficiency as energetic insult to skeletal muscle in chronic diseases.* J Cachexia Sarcopenia Muscle, 2018. **9**(5): p. 802-815.
137. Charles-Edwards, G., et al., *Effect of Iron Isomaltoside on Skeletal Muscle Energetics in Patients With Chronic Heart Failure and Iron Deficiency.* Circulation, 2019. **139**(21): p. 2386-2398.
138. Koves, T.R., et al., *Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance.* Cell Metab, 2008. **7**(1): p. 45-56.
139. Koves, T.R., et al., *Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency.* J Biol Chem, 2005. **280**(39): p. 33588-98.
140. Fingerhut, R., et al., *Hepatic carnitine palmitoyltransferase I deficiency: acylcarnitine profiles in blood spots are highly specific.* Clin Chem, 2001. **47**(10): p. 1763-8.
141. Ussher, J.R., et al., *The Emerging Role of Metabolomics in the Diagnosis and Prognosis of Cardiovascular Disease.* J Am Coll Cardiol, 2016. **68**(25): p. 2850-2870.
142. Konishi, M., et al., *Impact of Plasma Kynurenine Level on Functional Capacity and Outcome in Heart Failure- Results From Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF).* Circ J, 2016. **81**(1): p. 52-61.
143. Watanabe, Y., et al., *Stereospecificity of hepatic L-tryptophan 2,3-dioxygenase.* Biochem J, 1980. **189**(3): p. 393-405.
144. Wang, Q., et al., *Tryptophan-kynurenine pathway is dysregulated in inflammation, and immune activation.* Front Biosci (Landmark Ed), 2015. **20**: p. 1116-43.
145. Gibney, S.M., et al., *Poly I:C-induced activation of the immune response is accompanied by depression and anxiety-like behaviours, kynurenine pathway activation and reduced BDNF expression.* Brain Behav Immun, 2013. **28**: p. 170-81.
146. Laurans, L., et al., *Genetic deficiency of indoleamine 2,3-dioxygenase promotes gut microbiota-mediated metabolic health.* Nat Med, 2018. **24**(8): p. 1113-1120.
147. Metghalchi, S., et al., *Indoleamine 2,3-Dioxygenase Fine-Tunes Immune Homeostasis in Atherosclerosis and Colitis through Repression of Interleukin-10 Production.* Cell Metab, 2015. **22**(3): p. 460-71.
148. Melhem, N.J., et al., *Endothelial Cell Indoleamine 2, 3-Dioxygenase 1 Alters Cardiac Function After Myocardial Infarction Through Kynurenine.* Circulation, 2021. **143**(6): p. 566-580.

149. Haykowsky, M.J., et al., *Impaired aerobic capacity and physical functional performance in older heart failure patients with preserved ejection fraction: role of lean body mass.* J Gerontol A Biol Sci Med Sci, 2013. **68**(8): p. 968-75.
150. Edelmann, F., et al., *Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction: results of the Ex-DHF (Exercise training in Diastolic Heart Failure) pilot study.* J Am Coll Cardiol, 2011. **58**(17): p. 1780-91.
151. Pellicori, P., et al., *Global longitudinal strain in patients with suspected heart failure and a normal ejection fraction: does it improve diagnosis and risk stratification?* Int J Cardiovasc Imaging, 2014. **30**(1): p. 69-79.
152. Katz, S.D., et al., *Near-maximal fractional oxygen extraction by active skeletal muscle in patients with chronic heart failure.* J Appl Physiol (1985), 2000. **88**(6): p. 2138-42.
153. Brecht, A., et al., *Left Atrial Function in Preclinical Diastolic Dysfunction: Two-Dimensional Speckle-Tracking Echocardiography-Derived Results from the BEFRI Trial.* J Am Soc Echocardiogr, 2016. **29**(8): p. 750-758.
154. Badano, L.P., et al., *Standardization of left atrial, right ventricular, and right atrial deformation imaging using two-dimensional speckle tracking echocardiography: a consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging.* Eur Heart J Cardiovasc Imaging, 2018. **19**(6): p. 591-600.
155. Fontes-Carvalho, R., et al., *Left atrial deformation analysis by speckle tracking echocardiography to predict exercise capacity after myocardial infarction.* Rev Port Cardiol, 2018. **37**(10): p. 821-830.
156. Blume, G.G., et al., *Left atrial function: physiology, assessment, and clinical implications.* Eur J Echocardiogr, 2011. **12**(6): p. 421-30.
157. Kusunose, K., et al., *Independent association of left atrial function with exercise capacity in patients with preserved ejection fraction.* Heart, 2012. **98**(17): p. 1311-7.
158. Ponikowski, P., et al., *2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC.* Eur Heart J, 2016. **37**(27): p. 2129-2200.
159. Mondillo, S., et al., *Early detection of left atrial strain abnormalities by speckle-tracking in hypertensive and diabetic patients with normal left atrial size.* J Am Soc Echocardiogr, 2011. **24**(8): p. 898-908.

160. Welles, C.C., et al., *Left atrial function predicts heart failure hospitalization in subjects with preserved ejection fraction and coronary heart disease: longitudinal data from the Heart and Soul Study.* J Am Coll Cardiol, 2012. **59**(7): p. 673-80.
161. Frydas, A., et al., *Left atrial strain as sensitive marker of left ventricular diastolic dysfunction in heart failure.* ESC Heart Fail, 2020. **7**(4): p. 1956-1965.
162. Lundberg, A., et al., *Left atrial strain improves estimation of filling pressures in heart failure: a simultaneous echocardiographic and invasive haemodynamic study.* Clin Res Cardiol, 2019. **108**(6): p. 703-715.
163. Hanna, N., et al., *Differences in atrial versus ventricular remodeling in dogs with ventricular tachypacing-induced congestive heart failure.* Cardiovasc Res, 2004. **63**(2): p. 236-44.
164. Hughes, C.M., et al., *Nutritional intake and oxidative stress in chronic heart failure.* Nutr Metab Cardiovasc Dis, 2012. **22**(4): p. 376-82.
165. Anker, S.D., et al., *Elevated soluble CD14 receptors and altered cytokines in chronic heart failure.* Am J Cardiol, 1997. **79**(10): p. 1426-30.
166. Sandek, A., et al., *Studies on bacterial endotoxin and intestinal absorption function in patients with chronic heart failure.* Int J Cardiol, 2012. **157**(1): p. 80-5.
167. Sandek, A., et al., *Nutrition in heart failure: an update.* Curr Opin Clin Nutr Metab Care, 2009. **12**(4): p. 384-91.
168. Landi, F., et al., *Prevalence and potentially reversible factors associated with anorexia among older nursing home residents: results from the ULISSE project.* J Am Med Dir Assoc, 2013. **14**(2): p. 119-24.
169. Molfino, A., et al., *Cancer anorexia: hypothalamic activity and its association with inflammation and appetite-regulating peptides in lung cancer.* J Cachexia Sarcopenia Muscle, 2017. **8**(1): p. 40-47.
170. Martone, A.M., et al., *Anorexia of aging: a modifiable risk factor for frailty.* Nutrients, 2013. **5**(10): p. 4126-33.
171. Anker, S.D., et al., *Prognostic importance of weight loss in chronic heart failure and the effect of treatment with angiotensin-converting-enzyme inhibitors: an observational study.* Lancet, 2003. **361**(9363): p. 1077-83.
172. Lundy, R.F., Jr., et al., *Furosemide-induced food avoidance: evidence for a conditioned response.* Physiol Behav, 2004. **81**(3): p. 397-408.
173. Bayliss, J., et al., *Untreated heart failure: clinical and neuroendocrine effects of introducing diuretics.* Br Heart J, 1987. **57**(1): p. 17-22.

174. Brink, M., J. Wellen, and P. Delafontaine, *Angiotensin II causes weight loss and decreases circulating insulin-like growth factor I in rats through a pressor-independent mechanism.* J Clin Invest, 1996. **97**(11): p. 2509-16.

175. Landi, F., et al., *Anorexia, physical function, and incident disability among the frail elderly population: results from the ilSIRENTE study.* J Am Med Dir Assoc, 2010. **11**(4): p. 268-74.

176. Muscaritoli, M., et al., *Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics".* Clin Nutr, 2010. **29**(2): p. 154-9.

177. Kalantar-Zadeh, K., et al., *Appetite and inflammation, nutrition, anemia, and clinical outcome in hemodialysis patients.* Am J Clin Nutr, 2004. **80**(2): p. 299-307.

178. Clark, A.L., P.A. Poole-Wilson, and A.J. Coats, *Exercise limitation in chronic heart failure: central role of the periphery.* J Am Coll Cardiol, 1996. **28**(5): p. 1092-102.

179. Massie, B.M., et al., *Skeletal muscle metabolism during exercise under ischemic conditions in congestive heart failure. Evidence for abnormalities unrelated to blood flow.* Circulation, 1988. **78**(2): p. 320-6.

180. Krack, A., et al., *The importance of the gastrointestinal system in the pathogenesis of heart failure.* Eur Heart J, 2005. **26**(22): p. 2368-74.

181. Silverberg, D.S., *The role of erythropoiesis stimulating agents and intravenous (IV) iron in the cardio renal anemia syndrome.* Heart Fail Rev, 2011. **16**(6): p. 609-14.

182. Weiss, G. and L.T. Goodnough, *Anemia of chronic disease.* N Engl J Med, 2005. **352**(10): p. 1011-23.

183. Phan, T.T., et al., *Heart failure with preserved ejection fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency.* J Am Coll Cardiol, 2009. **54**(5): p. 402-9.

184. van Veldhuisen, D.J., et al., *Anemia and iron deficiency in heart failure: mechanisms and therapeutic approaches.* Nat Rev Cardiol, 2011. **8**(9): p. 485-93.

185. Ponikowski, P., et al., *Beneficial effects of long-term intravenous iron therapy with ferric carboxymaltose in patients with symptomatic heart failure and iron deficiencydagger.* Eur Heart J, 2015. **36**(11): p. 657-68.

186. Hughes, M.A., B.S. Myers, and M.L. Schenkman, *The role of strength in rising from a chair in the functionally impaired elderly.* J Biomech, 1996. **29**(12): p. 1509-13.

187. Macrae, P.G., M. Lacourse, and R. Moldavon, *Physical performance measures that predict faller status in community-dwelling older adults.* J Orthop Sports Phys Ther, 1992. **16**(3): p. 123-8.
188. Reichard, L.B., et al., *Testing knee extension and flexion strength at different ranges of motion: an isokinetic and electromyographic study.* Eur J Appl Physiol, 2005. **95**(4): p. 371-6.
189. Bento, P.C., et al., *Peak torque and rate of torque development in elderly with and without fall history.* Clin Biomech (Bristol, Avon), 2010. **25**(5): p. 450-4.
190. Lee, I.H. and S.Y. Park, *Balance improvement by strength training for the elderly.* J Phys Ther Sci, 2013. **25**(12): p. 1591-3.
191. McHugh, M.P., et al., *Differences in activation patterns between eccentric and concentric quadriceps contractions.* J Sports Sci, 2002. **20**(2): p. 83-91.
192. Cohen, J.B., et al., *Clinical Phenogroups in Heart Failure With Preserved Ejection Fraction: Detailed Phenotypes, Prognosis, and Response to Spironolactone.* JACC Heart Fail, 2020. **8**(3): p. 172-184.
193. Khayat, R., et al., *Sleep-disordered breathing in heart failure: identifying and treating an important but often unrecognized comorbidity in heart failure patients.* J Card Fail, 2013. **19**(6): p. 431-44.
194. Hanly, P. and N. Zuberi-Khokhar, *Daytime sleepiness in patients with congestive heart failure and Cheyne-Stokes respiration.* Chest, 1995. **107**(4): p. 952-8.
195. Bitter, T., et al., *Cheyne-Stokes respiration and obstructive sleep apnoea are independent risk factors for malignant ventricular arrhythmias requiring appropriate cardioverter-defibrillator therapies in patients with congestive heart failure.* Eur Heart J, 2011. **32**(1): p. 61-74.
196. Paulino, A., et al., *Prevalence of sleep-disordered breathing in a 316-patient French cohort of stable congestive heart failure.* Arch Cardiovasc Dis, 2009. **102**(3): p. 169-75.
197. Spaak, J., et al., *Muscle sympathetic nerve activity during wakefulness in heart failure patients with and without sleep apnea.* Hypertension, 2005. **46**(6): p. 1327-32.
198. Kaye, D.M., et al., *Adverse consequences of high sympathetic nervous activity in the failing human heart.* J Am Coll Cardiol, 1995. **26**(5): p. 1257-63.
199. Egerman, M.A. and D.J. Glass, *Signaling pathways controlling skeletal muscle mass.* Crit Rev Biochem Mol Biol, 2014. **49**(1): p. 59-68.

200. Naughton, M., et al., *Role of hyperventilation in the pathogenesis of central sleep apneas in patients with congestive heart failure.* Am Rev Respir Dis, 1993. **148**(2): p. 330-8.
201. White, L.H. and T.D. Bradley, *Role of nocturnal rostral fluid shift in the pathogenesis of obstructive and central sleep apnoea.* J Physiol, 2013. **591**(5): p. 1179-93.
202. Hanly, P., N. Zuberi, and R. Gray, *Pathogenesis of Cheyne-Stokes respiration in patients with congestive heart failure. Relationship to arterial PCO2.* Chest, 1993. **104**(4): p. 1079-84.
203. Leung, R.S., et al., *Provocation of ventricular ectopy by cheyne-stokes respiration in patients with heart failure.* Sleep, 2004. **27**(7): p. 1337-43.
204. Javaheri, S., *A mechanism of central sleep apnea in patients with heart failure.* N Engl J Med, 1999. **341**(13): p. 949-54.
205. van de Borne, P., et al., *Effect of Cheyne-Stokes respiration on muscle sympathetic nerve activity in severe congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy.* Am J Cardiol, 1998. **81**(4): p. 432-6.
206. Basner, R.C., *Continuous positive airway pressure for obstructive sleep apnea.* N Engl J Med, 2007. **356**(17): p. 1751-8.
207. Costanzo, M.R., et al., *Phrenic nerve stimulation to treat patients with central sleep apnoea and heart failure.* Eur J Heart Fail, 2018. **20**(12): p. 1746-1754.
208. Saint Martin, M., et al., *Association of body fat composition and obstructive sleep apnea in the elderly: A longitudinal study.* Obesity (Silver Spring), 2015. **23**(7): p. 1511-6.
209. Gavrilovic, B., M.R. Popovic, and A. Yadollahi, *Modeling sleep apnea severity using bioimpedance measurements.* Conf Proc IEEE Eng Med Biol Soc, 2015. **2015**: p. 5998-6001.
210. Redolfi, S., et al., *Relationship between overnight rostral fluid shift and Obstructive Sleep Apnea in nonobese men.* Am J Respir Crit Care Med, 2009. **179**(3): p. 241-6.
211. Koehler, F., et al., *Efficacy of telemedical interventional management in patients with heart failure (TIM-HF2): a randomised, controlled, parallel-group, unmasked trial.* Lancet, 2018. **392**(10152): p. 1047-1057.
212. Assaad, M., et al., *CardioMems(R) device implantation reduces repeat hospitalizations in heart failure patients: A single center experience.* JRSM Cardiovasc Dis, 2019. **8**: p. 2048004019833290.

213. Barker, W.H., J.P. Mullooly, and W. Getchell, *Changing incidence and survival for heart failure in a well-defined older population, 1970-1974 and 1990-1994.* Circulation, 2006. **113**(6): p. 799-805.

214. Lloyd-Jones, D., et al., *Executive summary: heart disease and stroke statistics--2010 update: a report from the American Heart Association.* Circulation, 2010. **121**(7): p. 948-54.

215. Bhatia, R.S., et al., *Outcome of heart failure with preserved ejection fraction in a population-based study.* N Engl J Med, 2006. **355**(3): p. 260-9.

216. Massie, B.M., et al., *Irbesartan in patients with heart failure and preserved ejection fraction.* N Engl J Med, 2008. **359**(23): p. 2456-67.

217. Yusuf, S., et al., *Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: the CHARM-Preserved Trial.* Lancet, 2003. **362**(9386): p. 777-81.

218. Bergstrom, A., et al., *Effect of carvedilol on diastolic function in patients with diastolic heart failure and preserved systolic function. Results of the Swedish Doppler-echocardiographic study (SWEDIC).* Eur J Heart Fail, 2004. **6**(4): p. 453-61.

219. Anker, S.D., et al., *Empagliflozin in Heart Failure with a Preserved Ejection Fraction.* N Engl J Med, 2021. **385**(16): p. 1451-1461.

220. Pandey, A., et al., *Exercise training in patients with heart failure and preserved ejection fraction: meta-analysis of randomized control trials.* Circ Heart Fail, 2015. **8**(1): p. 33-40.

221. Stevenson, L.W., et al., *Exercise capacity for survivors of cardiac transplantation or sustained medical therapy for stable heart failure.* Circulation, 1990. **81**(1): p. 78-85.

222. Maurer, M.S. and P.C. Schulze, *Exercise intolerance in heart failure with preserved ejection fraction: shifting focus from the heart to peripheral skeletal muscle.* J Am Coll Cardiol, 2012. **60**(2): p. 129-31.