ESC European Society of Cardiology

European Journal of Heart Failure (2022) **24**, 1940–1949
doi:10.1002/ejhf.2628

RESEARCH ARTICLE

Intravenous iron therapy improves the hypercapnic ventilatory response and sleep disordered breathing in chronic heart failure

Sergio Caravita[1,2†], Andrea Faini[1†], Carlo Vignati[3], Sara Pelucchi[4], Elisabetta Salvioni[3], Gaia Cattadori[5], Claudia Baratto[1], Camilla Torlasco[1], Mauro Contini[3], Alessandra Villani[1], Gabriella Malfatto[1], Elisa Perger[1], Carolina Lombardi[1,4], Alberto Piperno[4], Piergiuseppe Agostoni[3,6‡], and Gianfranco Parati[1,4*‡]

[1]Department of Cardiovascular, Neural and Metabolic Sciences, Istituto Auxologico Italiano IRCCS, Ospedale San Luca, Milan, Italy; [2]Department of Management, Information and Production Engineering, University of Bergamo, Dalmine, Italy; [3]Centro Cardiologico Monzino, IRCCS, Milan, Italy; [4]Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy; [5]MultiMedica IRCCS, Milan, Italy; and [6]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

Received 20 December 2021; revised 12 July 2022; accepted 20 July 2022; online publish-ahead-of-print 8 August 2022

Aims Intravenous iron therapy can improve symptoms in patients with heart failure, anaemia and iron deficiency. The mechanisms underlying such an improvement might involve chemoreflex sensing and nocturnal breathing patterns.

Methods and results Patients with heart failure, reduced left ventricular ejection fraction, anaemia (haemoglobin <13 g/dl in men; <12 g/dl in women) and iron deficiency (ferritin <100 or 100–299 μg/L with transferrin saturation <20%) were 2:1 randomized to patient-tailored intravenous ferric carboxymaltose dose or placebo. Chemoreflex sensitivity cardiorespiratory sleep study, symptom assessment and cardiopulmonary exercise test were performed before and 2 weeks after the last treatment dose. Fifty-eight patients (38 active arm/20 placebo arm) completed the study. Intravenous iron was associated with less severe symptoms, higher haemoglobin (12.5 ± 1.4 vs. 11.7 ± 1.0 mg/dl, $p < 0.05$) and improved haematinic parameters. Ferric carboxymaltose improved the central hypercapnic ventilatory response (−25.8%, $p < 0.05$ vs. placebo), without changes in peripheral chemosensitivity. In particular, the central hypercapnic ventilatory responses passed from 4.6 ± 6.5 to 2.9 ± 2.9 L/min/mmHg after ferric carboxymaltose and from 4.4 ± 4.6 to 4.6 ± 3.9 L/min/mmHg after placebo ($p_{treatment*condition} = 0.046$). In patients presenting with sleep-related breathing disorder, apnoea–hypopnoea index was reduced with active treatment as compared to placebo (12 ± 11 vs. 19 ± 13 events/h, $p < 0.05$). After ferric carboxymaltose, but not after placebo, both peak oxygen uptake (VO_2) increased ($\Delta 1.1 \pm 2.0$ ml/kg/min, $p < 0.05$) and VO_2/workload slope was steeper ($\Delta 0.67 \pm 1.7$ L/min/W, $p < 0.01$).

Conclusions Intravenous ferric carboxymaltose improves the hypercapnic ventilatory response and sleep-related breathing disorders in patients with heart failure, anaemia and iron deficiency. These newly described findings, along with improved oxygen delivery to exercising muscles, likely contribute to the favourable effects of ferric carboxymaltose in anaemic patients with heart failure.

Keywords Heart failure • Anaemia • Iron • Chemoreflex • Sleep • Exercise

*Corresponding author. Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy, Department of Cardiovascular, Neural and Metabolic Sciences, Istituto Auxologico Italiano IRCCS, Ospedale San Luca, Piazzale Brescia 20, 20149 Milan, Italy. Tel: +39 02 61911 2949/2890, Email: gianfranco.parati@unimib.it

†Equally contributed as first authors.

‡Equally contributed as last authors.

Background

Anaemia and disordered iron metabolism are ominous signs in chronic heart failure (HF).[1,2] Intravenous iron therapy improves several relevant clinical aspects,[3,4] including quality of life,[5–7] exercise capacity,[6,8] skeletal muscle energetics,[9] renal function[10] and prognosis[11] in symptomatic patients with HF and iron deficiency, with or without anaemia.[5,12] Accordingly, intravenous iron supplementation with ferric carboxymaltose should be considered in symptomatic HF patients with an ejection fraction ≤45% and iron deficiency, to alleviate symptoms, improve exercise capacity and quality of life.[13] However, the putative mechanisms accounting for such improvements in HF patients have been only partly clarified.[1]

Haemoglobin plays a pivotal role in oxygen transport and utilization, as well as in the clearance of carbon dioxide (CO_2), albeit this latter aspect is generally underappreciated.[13,14] Indeed, iron-deficient anaemia, by limiting both oxygen and CO_2 transport in the blood, might have deleterious effect on peripheral and/or central chemoreflex responses. Thus, we hypothesized that correction of iron-deficient anaemia by means of ferric carboxymaltose might improve chemoreflex sensing and patients' clinical status. Given the intricate link between chemoreflex sensitivity and deranged ventilation during sleep in patients with HF,[15] we also hypothesized that intravenous iron, by improving chemoreflex sensing, might reduce the burden of sleep disordered breathing in the subgroup of anaemic HF patients presenting with sleep apnoeas at baseline evaluation.

Therefore, in this double-blind study, patients with HF and iron-deficient anaemia were treated with ferric carboxymaltose or placebo, with the aim of specifically investigating the effect of such therapy on the ventilatory responses to hypoxia and hypercapnia, as well as on sleep disordered breathing. Complementarily, we also monitored the effects of ferric carboxymaltose on symptoms, blood tests (including markers of iron metabolism) and exercise performance.

Methods

The study was approved by the ethics committees of the Istituto Auxologico Italiano and the Centro Cardiologico Monzino and registered with the EudraCT number: 2012-005830-12. All enrolled patients signed a written informed consent to the study. In brief, in between 2013 and 2017, we included consecutive clinically stable patients with chronic HF that presented a left ventricular ejection fraction ≤45%, anaemia (haemoglobin 9–12 g/dl in women or 9–13 g/dl in men) and iron deficiency (serum ferritin <100 μg/L or 100–299 μg/L whether transferrin saturation was <20%). The complete list of inclusion and exclusion criteria is presented in the online data supplement.

Study treatment

Patients were randomized 2:1 to intravenous iron or placebo according to a centralized complete block design with six replications. Randomization was based on biased-coin minimization method and stratified by sex. The total dose of iron to be infused was calculated through Ganzoni's formula,[6] modified to take into account sex-related differences in haemoglobin values and to reduce the amount of iron calculated for deposits. Specifically, the following formula was applied: iron repletion dose (mg) = weight (kg) × 2.4 × [(15 for males, 13 for females) − haemoglobin (g/dl)] + 350. Calculated dose was rounded in excess.

Intravenous ferric carboxymaltose or saline placebo was administered every 3 weeks, with a maximum dose of 500 mg administered during each session, up to the completion of the calculated total dose. Because iron formulations are dark-brown and easily distinguishable from placebo, the whole infusion set was shielded from patient's sight, and the personnel responsible for the preparation and administration of the study drug was not involved in any study assessments. Safety criteria for intravenous iron administration are reported as online data supplement.

Study evaluations

At baseline (before treatment) as well as 2 weeks after completion of iron replenishment, patients underwent a thorough clinical assessment, including blood tests, standard echocardiography according to international recommendations,[16] cardiopulmonary exercise testing, chemoreflex sensitivity assessment, and cardiorespiratory sleep study. Cardiopulmonary exercise testing and chemoreflex sensitivity assessment were performed within 24 h, on two separate days.

Chemoreflex testing

Chemoreflex sensitivity assessment was performed according to previously published methodology,[17,18] as detailed below. Chemoreflex tests (peripheral chemosensitivity to hypoxia, peripheral chemosensitivity to hypercapnia and central chemosensitivity to hypercapnia) were always performed in the same order, separated by a 15-min interval. All tests were centrally and blindly reviewed by a single expert reader.

The patients were seated and connected with a mouthpiece to the V-MAX metabolic cart (Vmax SensorMedics 2200, Yorba Linda, CA, USA) in order to collect breath-by-breath data, including minute ventilation (VE) and end-expiratory partial pressure for CO_2 ($PetCO_2$). Peripheral oxygen saturation (SpO_2) was continuously measured with pulse oximeter and an ear probe. Calibration of the metabolic cart was performed before each test. For the assessment of peripheral hypoxic chemosensitivity and of peripheral chemosensitivity to CO_2, a non-rebreathing valve was placed in series to the mass flow sensor of the metabolic cart, which separated the inspirate from the expirate. The inspirate port was further connected to a T-valve shielded from patients' sight, and depending on the position of the T-valve, the subject breathed either room air or a gas mixture from a 4-L reservoir bag.

Peripheral hypoxic chemosensitivity

After several minutes of quite breathing, the T-valve was turned surreptitiously during the expiratory phase so that pure nitrogen was inhaled for two to eight breaths in a random fashion. This transient hypoxic challenge was repeated about 10–15 times in order to provide a wide range of SpO_2 from 70% to 100%, with at least 2-min interval between each test repetition, thus allowing SpO_2, $PetCO_2$ and VE to return at the individuals' baseline. The lowest SpO_2 reached after each nitrogen inhalation was plotted against the maximal VE (average of two consecutive breaths) obtained within 20 s from SpO_2 nadir. The regression slope between these two variables (VE/SpO_2 slope) was taken as an index of peripheral chemoreflex sensitivity to hypoxia,[17] and expressed in L/min/%.

Peripheral chemoreflex sensitivity to carbon dioxide

After the subject breathed room air for several minutes, the T-valve was turned surreptitiously during the expiratory phase so that the subject inhaled a single breath of 13% CO_2 in air. The mean VE and $PetCO_2$ of the five breaths before each CO_2 challenge were taken as control. The $PetCO_2$ of the stimulus breath was considered equal to the chemoreflex stimulus itself. The response in VE after the CO_2 challenge was calculated as the average of the two largest consecutive breaths within 20 s after CO_2 inhalation, thus assuming to exclude the ventilatory response determined by the central chemoreceptors. The single breath response was calculated as the ratio between delta VE and delta $PetCO_2$, and expressed in L/min/mmHg. The single breath hypercapnic challenge was repeated 10 times with at least 2-min interval in between each inhalation of 13% CO_2 in air, and the average of the single breath CO_2 response for each subject was taken as an index of peripheral chemoreflex sensitivity to CO_2.[17]

Central chemoreflex sensitivity

After the subject breathed room air for several minutes, they surreptitiously started a 4-min rebreathing from a 6-L bag containing 7% CO_2 in oxygen. With this technique, it is assumed that pCO_2 equilibrium is rapidly reached in the mixed venous blood, arterial blood, and gas in the breathing bag, and that with very high oxygen concentration the peripheral hypercapnic response would be minimal or negligible. The slope of the relationship between VE and $PetCO_2$, expressed in L/min/mmHg, was considered an index of central hypercapnic chemosensitivity.[17] Only the linear regression between the first identifiable increase in VE up to the end of the test, or up to plateauing of VE values towards the end of the test, was considered. Indeed, due to the quite long exposure times at the high CO_2 levels, individuals may be unable to sustain the high ventilation desired, or they may reach their maximum limit of ventilation. Eliminating the plateauing phase from the $VE/PetCO_2$ slope avoids factitious reductions of the hypercapnic ventilatory response.[19]

Cardiorespiratory sleep studies

Cardiorespiratory sleep studies were performed with a standard device (Embletta, Embla, Broomfield, CO, USA). All tests were centrally reviewed and scored by a single blinded expert reader, according to international guidelines,[20] identifying sleep period based on what reported by the patient in the sleep diary. The presence of sleep disordered breathing was defined by the presence of an apnoea–hypopnoea index (AHI) >5 events per hour of sleep. Thus, for the evaluation of the effect of ferric carboxymaltose (or of placebo) on sleep disordered breathing, individuals with baseline AHI ≤5/h were discarded from cardiorespiratory sleep study analysis.

Cardiopulmonary exercise test

A symptom-limited cardiopulmonary exercise testing was conducted on a cycle ergometer, according to a personalized ramp incremental protocol (ranging from 5 to 15 W/min), aimed at achieving maximal exercise capacity in 8–12 min.[21] Both the recruiting centers (Ospedale San Luca IRCCS Istituto Auxologico Italiano and Centro Cardiologico Monzino) implemented the same methodology and instrumentation, analysing breath-by-breath data with V-MAX metabolic cart (Vmax SensorMedics 2200, Yorba Linda, CA, USA). All tests were centrally and blindly reviewed by a single expert reader. The average of the last 30-s exercise time was taken as an estimate of the peak value for the variables of interest. The first ventilatory threshold was calculated by the V-slope method and confirmed by analysis of the end-tidal pressures plot and the ventilatory equivalents plot. The VE/VCO_2 slope was calculated over the linear component of VE versus VCO_2.[22]

Statistics and sample size considerations

Quantitative variables are expressed as mean ± standard deviation unless otherwise specified, whilst categorical variables as an absolute number (percentage). We employed the two-tailed *t*-test, when applicable, or a two-tailed Mann–Whitney U test for continuous data and the chi-squared or the Fisher exact test, according to the sample size and cell count, for categorical variables. We used linear mixed-effects models accounting for repeated measurements with an unstructured covariance matrix, fitting the models by maximizing the restricted log-likelihood followed by a posteriori contrasts when applicable. The false discovery rate algorithm was used for multiple post-hoc comparisons. The variables were transformed to handle possible violations of the hypothesis of normality of the residuals. An α-level of 0.05 was used for all hypothesis tests; analyses were performed using R Core Team software (Vienna, Austria).

Based on previous data,[17,18] we estimated that 66 individuals (60 with a 10% dropout) would have been enough to demonstrate, with a $p < 0.05$ and a power of 0.8, an improvement by 0.1 L/min/% of peripheral chemosensitivity, and by 0.5 L/min/mmHg of central chemosensitivity, as a result of treatment. Due to a higher dropout rate than expected, enrolment was continued up to including 70 patients. This sample size was similarly expected to be enough to explore potential differences also in other meaningful pathophysiological variables, such as AHI at polysomnography and peak oxygen uptake (VO_2) at cardiopulmonary exercise test.

Results

Patients' flow is depicted in *Figure 1*. Out of 70 enrolled patients, 47 were assigned to active treatment and 23 to placebo. Fifty-eight patients (38 randomized to ferric carboxymaltose and 20 to placebo) completed the treatment phase and were subsequently re-assessed. The treatment was well tolerated without side effects in both study arms. The median (interquartile range) interval in between the first evaluation and the post-treatment assessment was 51 (47–64) and 50 (42–61) days in the active and placebo group, respectively.

General characteristics

At baseline, there were no between-groups differences in general clinical characteristics (*Table 1*). The leading aetiology of HF was ischaemic heart disease (69% of patients), mean left ventricular ejection fraction was 35 ± 8% and mean estimated systolic pulmonary artery pressure was 41 ± 14 mmHg. Mean haemoglobin value was 11.4 ± 1.0 g/dl, with a mean estimated iron need of 1.0 ± 0.3 g. Fifty-five percent of patients were in New York Heart Association (NYHA) class III. This corresponded to neurohumoral derangement (B-type natriuretic peptide, median [interquartile range]: 345 [207–756] ng/L) coupled with a quite severe functional impairment at cardiopulmonary exercise test, as witnessed by a

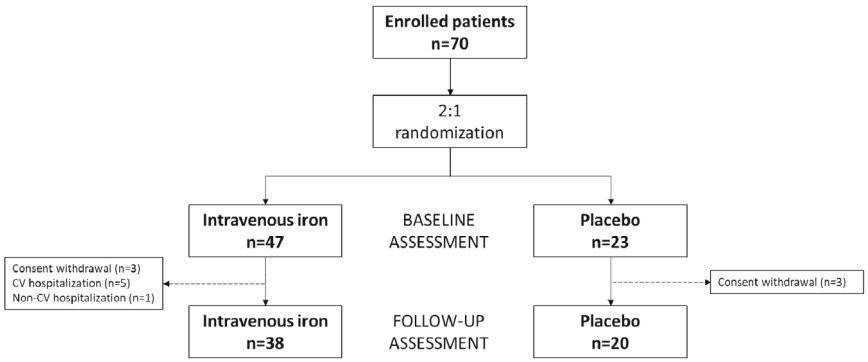

Figure 1 Patients' flow. CV, cardiovascular.

mean peakVO_2 of 13.6 ± 3.9 ml/kg/min and a mean VE/VCO_2 slope of 33 ± 7.

Blood tests

Intravenous iron therapy, but not placebo, was associated to a significant increase in haemoglobin and haematocrit (*Table 2*). This was paralleled by an increase in ferritin, transferrin saturation, hepcidin, and by a reduction of soluble transferrin receptor (*Table 2*). Fifty-one percent of patients in the active arm group and only one patient in the placebo group had a haemoglobin increase >1 g/dl as an effect of treatment.

Chemoreflex sensitivity

Before treatment, there were no significant differences in chemoreflex sensitivity between the active arm and the placebo group (*Figure 2* and online supplementary *Table S1*), albeit with wide variability of individual responses (online supplementary *Figures S1* and *S2*). As a result of treatment, patients randomized to intravenous iron therapy, as compared to those randomized to placebo, presented a significant reduction ($p = 0.023$) of the VE/PetCO_2 slope, suggesting a reduction of central chemoreflex sensitivity to CO_2 (*Figure 3*). Results remained consistent even after removing from the active treatment group two potential 'outliers' characterized by greatly enhanced response before treatment and markedly reduced response after treatment ($p_{treatment*condition} = 0.048$). Neither intravenous iron therapy nor placebo was associated with changes in peripheral chemoreflex sensitivity to either hypoxia or hypercapnia (*Figure 3*, online supplementary *Table S1*, *Figures S1* and *S2*).

Sleep disordered breathing

Sleep studies of adequate quality were obtained in 83% of patients at baseline and in 86% of patients after treatment. Thirty-four individuals (21 active arm, 13 placebo) presented with sleep disordered breathing at baseline (AHI >5/h). In this subset of patients with AHI >5/h, mean AHI was 21.4 ± 16.0 in the active arm group and 20.6 ± 14.7 in the placebo group, with no between-groups differences. After treatment, intravenous iron therapy resulted in lower

Table 1 General characteristics of the study population

	Iron ($n = 38$)	Placebo ($n = 20$)	*p*-value
Demographics and anthropometrics			
Age, years	71 ± 10	71 ± 10	0.990
Female sex	8 (21)	4 (20)	>0.99
Weight, kg	74 ± 18	79 ± 20	0.462
Height, cm	169 ± 9	171 ± 10	0.451
BMI, kg/m^2	26.0 ± 4.6	26.8 ± 5.2	0.540
Aetiology of heart failure			0.890
Cardiomyopathy	10 (26)	6 (30)	
Ischaemic heart disease	27 (71)	13 (65)	
Valvular heart disease	1 (3)	1 (5)	
Rhythm			
Atrial fibrillation	8 (21)	7 (35)	0.402
Pacemaker rhythm	21 (55)	13 (65)	0.663
Bood tests			
Creatinine, mg/dl	1.4 ± 0.5	1.5 ± 0.6	0.546
BNP, ng/L	638 ± 798	549 ± 490	0.317
Echocardiography			
LVEDV, ml	179 ± 67	179 ± 53	0.488
LVEF, %	35 ± 7	35 ± 8	0.834
sPAP, mmHg	40 ± 13	42 ± 16	0.840
Treatment			
Diuretics	34 (94)	15 (88)	0.586
ACE-inhibitors	21 (55)	10 (50)	0.916
ARBs	12 (32)	5 (25)	0.826
Beta-blockers	34 (90)	20 (100)	0.338
MRAs	20 (53)	11 (55)	>0.99
Amiodarone	22 (58)	9 (45)	0.510
Antiplatelet drugs	26 (68)	12 (60)	0.726
Oral anticoagulants	16 (42)	9 (45)	>0.99
ICD	23 (61)	16 (80)	0.227
CRT	12 (32)	6 (30)	>0.99

Values are given as mean ± standard deviation, or *n* (%).
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BNP, B-type natriuretic peptide; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter defibrillator; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; sPAP, systolic pulmonary artery pressure.

Table 2 Blood and haematinic parameters in the study population, before and after treatment

	Treatment arm	Before treatment	After treatment	$p_{treatment}$	$p_{condition}$	$p_{treatment*condition}$
RBCs, $\times 10^{12}$/L	Iron	4.1 ± 0.4	4.3 ± 0.6*	0.268	**0.023**	0.491
	Placebo	4.3 ± 0.8	4.4 ± 0.8			
Haemoglobin, g/dl	Iron	11.4 ± 1.1	12.5 ± 1.4***†	0.214	**<0.001**	**0.020**
	Placebo	11.4 ± 1.1	11.7 ± 1.0			
Haematocrit, %	Iron	36 ± 3	38 ± 4***	0.384	**0.001**	**0.034**
	Placebo	36 ± 3	36 ± 3			
Mean globular volume, fl	Iron	87 ± 8	90 ± 5***†	0.067	**<0.001**	**<0.001**
	Placebo	84 ± 11	84 ± 11			
Mean globular haemoglobin, pg	Iron	28.1 ± 3.5	29.7 ± 2.0***†	0.059	**<0.001**	**<0.001**
	Placebo	27.1 ± 4.1	27.1 ± 4.3			
RBC distribution width, %	Iron	16.1 ± 2.1	18.1 ± 3.7***	0.626	**0.032**	**<0.001**
	Placebo	16.9 ± 1.5	16.8 ± 2.0			
Iron, mg/dl	Iron	50 ± 23	72 ± 25***	0.700	**<0.001**	**0.015**
	Placebo	56 ± 22	62 ± 25			
Transferrin, mg/dl	Iron	293 ± 60	236 ± 38***†	**0.003**	**<0.001**	**<0.001**
	Placebo	296 ± 39	305 ± 46			
Transferrin saturation, %	Iron	13 ± 6	22 ± 8***†††	**0.041**	**<0.001**	**0.001**
	Placebo	14 ± 6	15 ± 7			
Ferritin, µg/L	Iron	59 ± 63	286 ± 222***†††	**<0.001**	**<0.001**	**<0.001**
	Placebo	54 ± 38	70 ± 131			
Hepcidin, ng/ml	Iron	15.8 ± 13.1	46.7 ± 53.6**†	**0.022**	0.150	**0.019**
	Placebo	8.9 ± 7.6	7.6 ± 8.1			
sTFR, ng/ml	Iron	33.8 ± 17.9	22.9 ± 8.6***†	0.247	**0.008**	**<0.001**
	Placebo	31.5 ± 13.4	32.9 ± 12.7			

sTFR, soluble transferrin receptor; RBC, red blood cell.
*$p < 0.05$ vs. pre-treatment; **$p \leq 0.01$ vs. pre-treatment; ***$p \leq 0.001$ vs. pre-treatment; †$p < 0.05$ vs. placebo; ††$p \leq 0.01$ vs. placebo; †††$p \leq 0.001$ vs. placebo.

AHI, as compared with placebo (12 ± 11 vs. 19 ± 13, $p < 0.05$). The distribution of central, obstructive, and mixed sleep apnoeas, as well as of hypopnoeas in these two groups before and after treatment is shown in *Figure 4*.

Functional capacity

Patients treated by intravenous iron, as compared with those on placebo, improved symptoms and functional capacity (online supplementary *Table S2*). The prevalence of a NYHA class >II was 61% and 45% of the active and placebo arm at baseline, and shifted to 26% and 50%, respectively, after treatment ($p_{treatment} = 0.143$, $p_{condition} = 0.063$, $p_{treatment*condition} = 0.020$). In patients treated with intravenous iron, peak VO_2 improved by 1.1 ± 2.0 ml/kg/min ($p = 0.027$) while it did not show any relevant change (0.3 ± 2.3 ml/kg/min) on placebo. Similarly, VO_2/work slope was higher in the active than in the placebo arm after treatment ($p = 0.009$).

Discussion

Our study, conducted with a rigorous double-blind methodology, suggests that correction of iron-deficient anaemia with ferric carboxymaltose may improve the central hypercapnic ventilatory response to rebreathing, as an index of central chemoreflex sensitivity, in patients with HF. Additionally, our data suggest that iron treatment reduces AHI in anaemic, iron-deficient HF patients with sleep-related breathing disorders. Finally, our study confirms the significant albeit modest beneficial role of intravenous iron supplementation in exercise capacity in this population.

Chemoreflex sensitivity has a pivotal role in several aspects of the complex pathophysiology of the HF syndrome, having been associated with more severe symptoms, as well as with markers of disease severity,[15,16] including sleep disordered breathing, and poor prognosis.[23] However, evaluation of chemoreflex sensitivity has not been widely standardized, results might differ based on the methodology employed,[19] and there might be wide inter-individual variability of responses.[18] Additionally, it has been suggested that the prevalence and clinical consequences of the autonomic derangement (including central chemoreceptor hypersensitivity) might have been changing over the past decade.[24] Nonetheless, if we compare our results to those obtained with an identical methodology to assess chemoreflex testing, some aspects deserve mention. First, as compared with a recent study in a contemporary, non-anaemic HF cohort where the effect of different beta-blockers on the chemoreflex were tested,[18] our patients displayed a similar peripheral chemoreflex response to both hypoxia and hypercapnia, but a higher chemoreflex sensitivity to hypercapnia (on average, 4.6 L/min/mmHg before and 2.9 L/min/mmHg after ferric carboxymaltose in our study vs. mean values in between

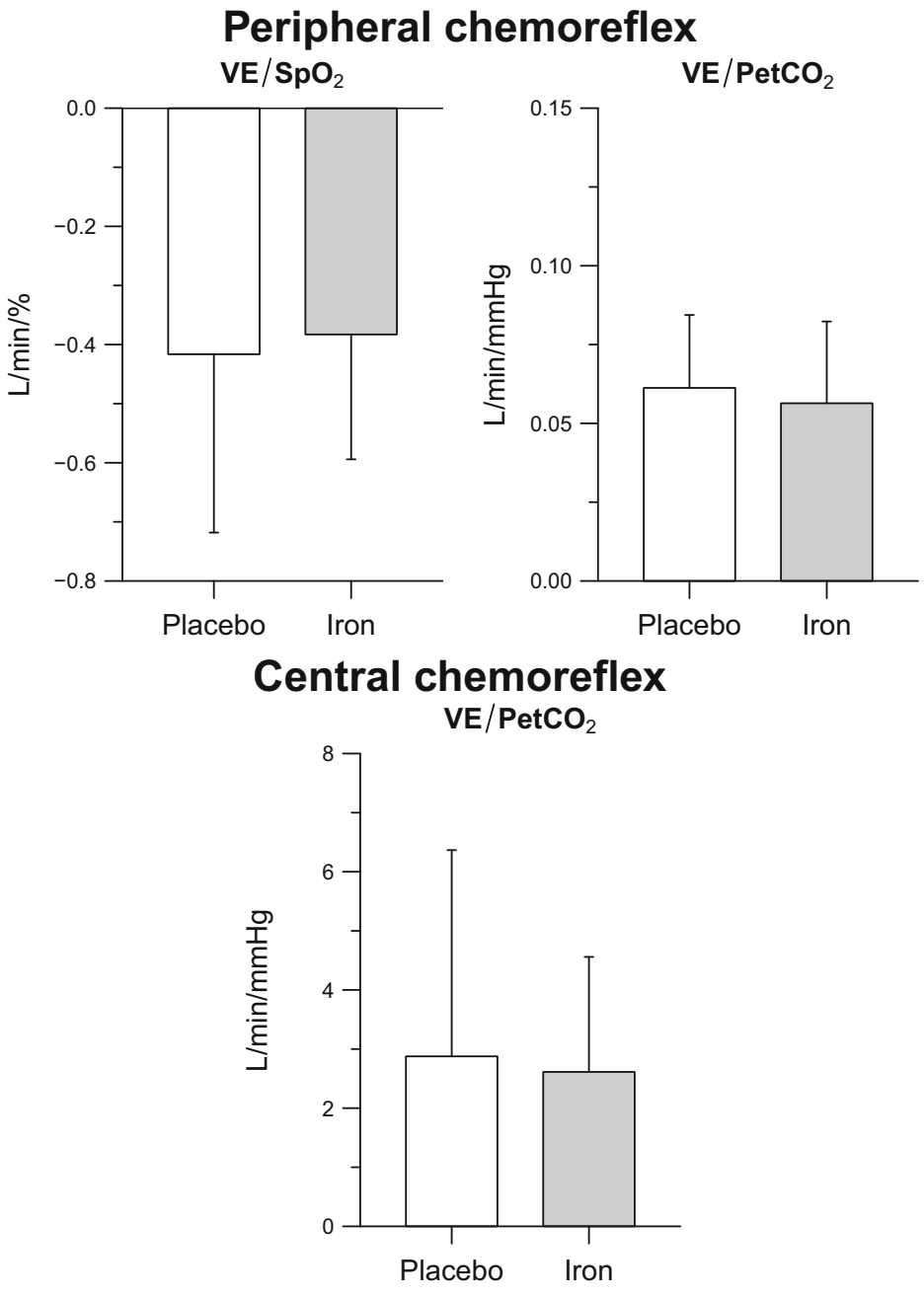

Figure 2 Baseline chemoreflex sensitivity in the two study groups. There were no differences in chemoreflex sensitivity between the two study groups at baseline. Values are expressed as median and median absolute deviation. $PetCO_2$, partial end-tidal pressure for carbon dioxide; SpO_2, peripheral oxygen saturation; VE, minute ventilation. *$p < 0.05$.

2.7 L/min/mmHg on carvedilol to 3.1 L/min/mmHg on bisoprolol in the study by Contini *et al.*[18]). This observation indirectly suggests that iron-deficient anaemia could negatively affect CO_2 sensing, activating the central chemoreflex. Additionally, the reduction of the central hypercapnic ventilatory response after intravenous iron administration in our HF patients suggests a potential reversibility of such alteration after correction of anaemia. A potential explanation for this finding relies on the pivotal role that haemoglobin plays in the removal of CO_2 from the periphery to the lung and its transfer to the alveoli.[14,25] Indeed, only 10% of CO_2 is dissolved in the blood. Another 10% is directly bound to haemoglobin to form carbamate but, most importantly, the great majority of CO_2 is transported in the blood in the form of HCO_3^-, the formation of which is catalysed by carbonic anhydrase within the red blood cell, thanks to the large oxylable buffering capacity of haemoglobin.[14,25,26] Thus, a reduction or sequestration of iron

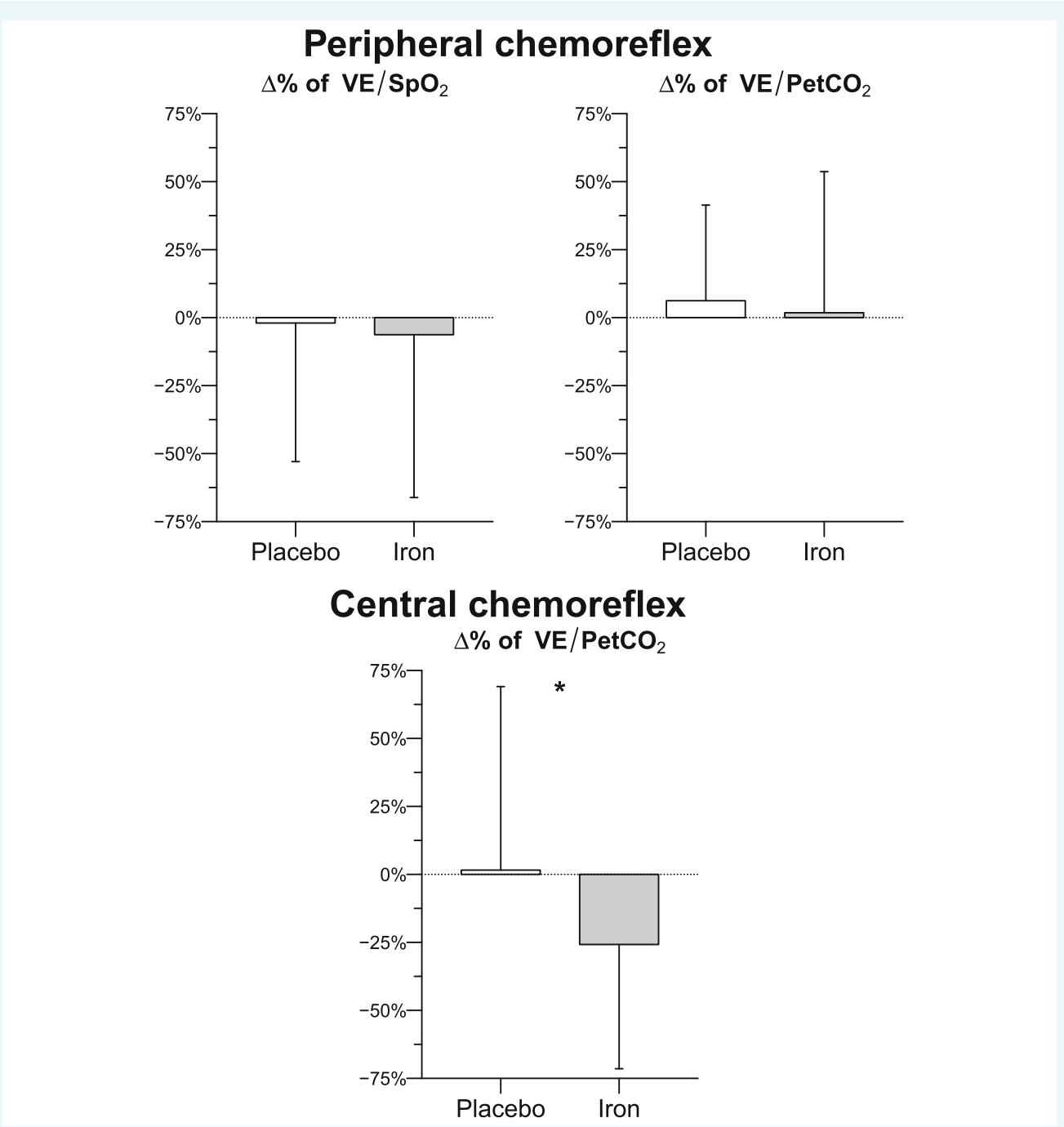

Figure 3 Percent variations in chemoreflex sensitivity after treatment in the two study groups. Peripheral chemoreflex sensitivity, both to oxygen and to carbon dioxide did not change in either group as a result of treatment. The central ventilatory response to carbon dioxide was improved (reduced) only in patients treated with intravenous iron. Values are expressed as median and median absolute deviation. $PetCO_2$, partial end-tidal pressure for carbon dioxide; SpO_2, peripheral oxygen saturation; VE, minute ventilation. *$p < 0.05$.

stores not available for the erythroid pool, eventually resulting in anaemia, will reduce the CO_2 clearance capacity of blood. In this context, patients with HF might not be able to implement the several compensatory mechanisms to maintain CO_2 output described in experimental animal models of acute anaemia,[14] including an increase of cardiac output and a further utilization of the Haldane effect, already maximalized by HF itself. The resultant increase of the hypercapnic ventilatory response during CO_2 rebreathing, either representing an activation of the central chemoreflex or, more likely, an increase of the intensity of the stimulus to central chemoreceptors, could be reverted by ferric carboxymaltose supplementation and correction of anaemia.

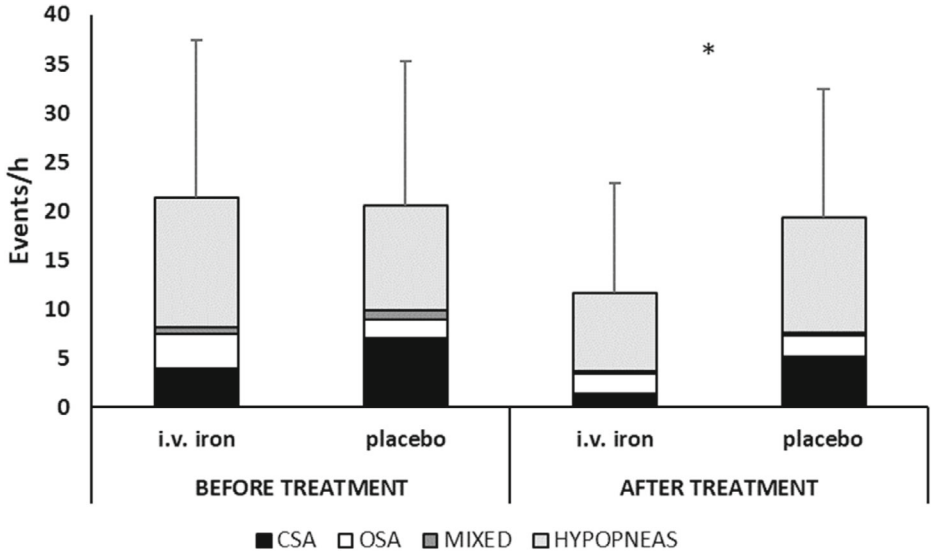

Figure 4 Burden of sleep disordered breathing before and after treatment in the two study groups. The distribution of central, obstructive, mixed apnoeas as well as hypopnoeic events is shown. CSA, central sleep apnoea; i.v., intravenous; mixed, mixed sleep apnoea; OSA, obstructive sleep apnoea. *$p < 0.05$.

Notably, and coherently to the modulation of the ventilatory response to CO_2, also the AHI was reduced after ferric carboxymaltose in our anaemic HF patients, confirming previous findings obtained in HF patients treated with a combination of erythropoietin and intravenous iron.[27] Indeed, the ventilatory response to hypercapnia has been associated with sleep-related breathing disorders: mainly of the central type[15] but also the obstructive one.[28] Thus, collectively our results suggest a novel mechanism, dependent on central chemoreflex modulation, accounting for symptoms and sleep-related breathing alterations in iron-deficient HF patients.

Somehow counterintuitively, correction of anaemia by ferric carboxymaltose did not affect peripheral chemoreflex sensing to oxygen in our study, although an indirect demonstration of tonic activation of the peripheral chemoreflex in anaemic HF patients has been previously provided by Franchitto *et al.*[29] In particular, in 36 HF patients, Franchitto *et al.* could show that haemoglobin was weakly correlated with muscle sympathetic nerve activity, and that acute peripheral chemoreflex deactivation with 100% oxygen was associated with a reduction of sympathetic discharge only in the 18 anaemic HF patients.[29] The same results were thereafter replicated in 15 patients with cardiorenal anaemia syndrome and 15 HF controls.[29] However, in those studies, patients' iron status was unknown, and conclusions were drawn based on acute chemoreflex deactivation rather than by correcting anaemia, and direct chemoreflex testing including the ventilatory responses to hypoxia was not obtained. Moreover, the effect of 100% oxygen breathing on the chemoreflex could be notably higher than that obtained through ferric carboxymaltose, which increased haemoglobin by 1 g/dl on average in our patients. Furthermore, we cannot exclude that a potential positive effect on the peripheral chemoreflex in our anaemic HF patients could have been speculatively blunted by oxidative stress,[30] this latter favoured by intravenous iron itself.

Finally, and coherently with previous studies on intravenous iron therapy in HF patients, we could demonstrate a small but not negligible improvement of peak VO_2 in actively treated patients,[8,31] which was associated with a slightly steeper VO_2/work slope. The latter is likely due to an increase of cardiac contractile reserve, of cardiac output response to exercise, and of oxygen delivery to exercising muscles.

Study limitations

Since we enrolled only a relatively small group of anaemic, iron-deficient HF patients, we cannot determine whether the improvement in central chemoreflex sensitivity is a direct consequence of iron replenishment or whether (more likely) it is mediated by haemoglobin improvement. Further studies might be needed to verify the efficacy of intravenous iron therapy in non-anaemic, iron-deficient patients.

We roughly estimated central chemoreflex sensitivity by the slope between $PetCO_2$ and minute ventilation, in analogy to previous investigations.[17,18] However, $PetCO_2$ does not correspond to medullary CO_2. Additionally, as discussed, increased clearance of CO_2 as a consequence of increased haemoglobin might determine a more efficient lung gas exchange in spite of unaltered chemoreflex sensitivity. If CO_2 was more efficiently cleared from tissues after ferric carboxymaltose as a function of higher haemoglobin, the rate of rise in end-tidal CO_2 might not have been equivalent to that of medullary pCO_2, nonetheless leading to a less sharp ventilatory response to CO_2 in spite of unchanged sensitivity of the central chemoreflex. Additionally, since we did not use the Duffin's modified rebreathing technique including prior hyperventilation, (i)

we could not determine the CO_2 threshold,[19] and (ii) we cannot exclude that full equilibration of mixedvenous and end-tidal partial pressures of CO_2 might have not occurred, occasionally leading to steeper or shallower slopes in a given patient.[19] Finally, chemoreflex responses display a great inter- and intra-individual variability,[32] with the potential for some outliers to relevantly affect between-groups statistical comparisons.

The VE/VCO_2 slope did not significantly change after intravenous iron therapy, despite the improvement in central chemoreflex. However, central chemoreflex is only one of the actors influencing ventilation during exercise, which is under the control of several mechanisms, including peripheral chemoreceptors, lung stretch receptors, lung stiffness, ventilation/perfusion matching.[33–35]

Albeit we could demonstrate a reduction of apnoeas/hypopnoeas per hour of sleep as an effect of intravenous iron in our patients, our study was not adequately powered to detect a statistically significant difference in a specific type of apnoeas. However, given the role of central chemoreflex sensitivity in regulating the CO_2 ventilatory (apnoeic) threshold and consequently in determining central sleep apnoea, we could suppose that the reduction in AHI might be mainly due to the favourable effect on central type alterations during sleep. Additionally, we should acknowledge that portable cardiorespiratory polygraph for diagnosis of sleep-related breathing disorders might over- or underestimate sleep apnoea in certain subject groups. However, they are routinely used in the clinical setting, in combination with a sleep diary to identify the actual patient's sleep time, as done also in our study. Finally, the study was conducted before sacubitril/valsartan became largely available in clinical practice for the treatment of HF with reduced ejection fraction. However, we do not believe that routine use of sacubitril/valsartan would have relevantly changed our results, nor this limitation would lessen their importance.

Conclusions

Our data suggest that intravenous ferric carboxymaltose may reduce the hypercapnic ventilatory response in anaemic, iron-deficient, low ejection fraction HF patients. Additionally, it could improve AHI in those patients presenting with sleep disordered breathing. These newly described effects, along with better peripheral oxygen delivery, can contribute to explain the global clinical improvement observed in anaemic HF patients after proper iron replenishment.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Acknowledgements

The authors would like to thank Caterina Bonino, MD; Giovanna Branzi, MD; Francesca Ciambellotti, MD; Antonella Dubini, MSc; Dario Pellegrini, MD; Miriam Revera, MD, PhD; Gino Luciano Seravalle, MD; Antonio Sorropago, MD; and Elena Viganò, MD (Istituto Auxologico Italiano IRCCS, Ospedale San Luca) for their help in data collection and/or data analysis. Open access funding provided by BIBLIOSAN.

Funding

The study was funded by the Italian Ministry of Health (RF-2010-2321799).

Conflict of interest: none declared.

References

1. Anand IS, Gupta P. Anemia and iron deficiency in heart failure: current concepts and emerging therapies. *Circulation*. 2018;**138**:80–98.
2. Jankowska EA, Rozentryt P, Witkowska A, Nowak J, Hartmann O, Ponikowska B, et al. Iron deficiency: an ominous sign in patients with systolic chronic heart failure. *Eur Heart J*. 2010;**31**:1872–80.
3. Rocha BML, Cunha GJL, Menezes Falcão LF. The burden of iron deficiency in heart failure: therapeutic approach. *J Am Coll Cardiol*. 2018;**71**:782–93.
4. von Haehling S, Ebner N, Evertz R, Ponikowski P, Anker SD. Iron deficiency in heart failure: an overview. *JACC Heart Fail*. 2019;**7**:36–46.
5. Comin-Colet J, Lainscak M, Dickstein K, Filippatos GS, Johnson P, Lüscher TF, et al. The effect of intravenous ferric carboxymaltose on health-related quality of life in patients with chronic heart failure and iron deficiency: a subanalysis of the FAIR-HF study. *Eur Heart J*. 2013;**34**:30–8.
6. Anker SD, Comin Colet J, Filippatos G, Willenheimer R, Dickstein K, Drexler H, et al.; FAIR-HF Trial Investigators. Ferric carboxymaltose in patients with heart failure and iron deficiency. *N Engl J Med*. 2009;**361**:2436–48.
7. Ponikowski P, van Veldhuisen DJ, Comin-Colet J, Ertl G, Komajda M, Mareev V, et al.; CONFIRM-HF Investigators. Beneficial effects of long-term intravenous iron therapy with ferric carboxymaltose in patients with symptomatic heart failure and iron deficiency. *Eur Heart J*. 2015;**36**:657–68.
8. Okonko DO, Grzeslo A, Witkowski T, Mandal AK, Slater RM, Roughton M, et al. Effect of intravenous iron sucrose on exercise tolerance in anemic and nonanemic patients with symptomatic chronic heart failure and iron deficiency: FERRIC-HF: a randomized, controlled, observer-blinded trial. *J Am Coll Cardiol*. 2008;**51**:103–12.
9. Charles-Edwards G, Amaral N, Sleigh A, Ayis S, Catibog N, McDonagh T, et al. Effect of iron isomaltoside on skeletal muscle energetics in patients with chronic heart failure and iron deficiency. *Circulation*. 2019;**21**:2386–98.
10. Ponikowski P, Filippatos G, Colet JC, Willenheimer R, Dickstein K, Lüscher T, et al.; FAIR-HF Trial Investigators. The impact of intravenous ferric carboxymaltose on renal function: an analysis of the FAIR-HF study. *Eur J Heart Fail*. 2015;**17**:329–39.
11. Anker SD, Kirwan BA, van Veldhuisen DJ, Filippatos G, Comin-Colet J, Ruschitzka F, et al. Effects of ferric carboxymaltose on hospitalisations and mortality rates in iron-deficient heart failure patients: an individual patient data meta-analysis. *Eur J Heart Fail*. 2018;**20**:125–33.
12. Filippatos G, Farmakis D, Colet JC, Dickstein K, Lüscher TF, Willenheimer R, et al. Intravenous ferric carboxymaltose in iron-deficient chronic heart failure patients with and without anaemia: a subanalysis of the FAIR-HF trial. *Eur J Heart Fail*. 2013;**15**:1267–76.
13. TA MD, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). With the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail*. 2022;**24**:4–131.
14. Deem S, Alberts MK, Bishop MJ, Bidani A, Swenson ER. CO_2 transport in normovolemic anemia: complete compensation and stability of blood CO_2 tensions. *J Appl Physiol (1985)*. 1997;**83**:240–6.
15. Giannoni A, Emdin M, Poletti R, Bramanti F, Prontera C, Piepoli M, et al. Clinical significance of chemosensitivity in chronic heart failure: influence on neurohormonal derangement, Cheyne-Stokes respiration and arrhythmias. *Clin Sci (Lond)*. 2008;**114**:489–97.
16. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the

European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging*. 2015;**16**:233–70.
17. Chua TP, Clark AL, Amadi AA, Coats AJ. Relation between chemosensitivity and the ventilatory response to exercise in chronic heart failure. *J Am Coll Cardiol*. 1996;**27**:650–7.
18. Contini M, Apostolo A, Cattadori G, Paolillo S, Iorio A, Bertella E, et al. Multiparametric comparison of CARvedilol, vs. NEbivolol, vs. BIsoprolol in moderate heart failure: the CARNEBI trial. *Int J Cardiol*. 2013;**168**:2134–40.
19. Mohan RM, Amara CE, Cunningham DA, Duffin J. Measuring central-chemoreflex sensitivity in man: rebreathing and steady-state methods compared. *Respir Physiol*. 1999;**115**:23–33.
20. Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, et al. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. *J Clin Sleep Med*. 2012;**8**:597–619.
21. Agostoni P, Bianchi M, Moraschi A, Palermo P, Cattadori G, La Gioia R, et al. Work-rate affects cardiopulmonary exercise test results in heart failure. *Eur J Heart Fail*. 2005;**7**:498–504.
22. Agostoni P, Dumitrescu D. How to perform and report a cardiopulmonary exercise test in patients with chronic heart failure. *Int J Cardiol*. 2019;**288**:107–13.
23. Giannoni A, Emdin M, Bramanti F, Iudice G, Francis DP, Barsotti A, et al. Combined increased chemosensitivity to hypoxia and hypercapnia as a prognosticator in heart failure. *J Am Coll Cardiol*. 2009;**53**:1975–80.
24. Paleczny B, Olesińska M, Siennicka A, Niewiński P, Jankowska EA, Ponikowska B, et al. Central chemoreceptor sensitivity is not enhanced in contemporary patients with chronic systolic heart failure receiving optimal treatment. *J Card Fail*. 2017;**23**:83–7.
25. Doyle J, Cooper JS Physiology, carbon dioxide transport. 2021 Jul 12. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan. PMID: 30422582.
26. Klocke RA. Carbon dioxide transport. Handbook of Physiology. The Respiratory System. Gas Exchange, sect. 3, vol. IV, chapt. 10. Bethesda, MD: American Physiological Society; 1987. p. 173–97.
27. Zilberman M, Silverberg DS, Bits I, Steinbruch S, Wexler D, Sheps D, et al. Improvement of anemia with erythropoietin and intravenous iron reduces sleep-related breathing disorders and improves daytime sleepiness in anemic patients with congestive heart failure. *Am Heart J*. 2007;**154**:870–6.
28. Lombardi C, Meriggi P, Agostoni P, Faini A, Bilo G, Revera M, et al.; HIGHCARE Investigators. High-altitude hypoxia and periodic breathing during sleep: gender-related differences. *J Sleep Res*. 2013;**22**:322–30.
29. Franchitto N, Despas F, Labrunée M, Roncalli J, Boveda S, Galinier M, et al. Tonic chemoreflex activation contributes to increased sympathetic nerve activity in heart failure-related anemia. *Hypertension*. 2010;**55**:1012–7.
30. Ding Y, Li YL, Zimmerman MC, Schultz HD. Elevated mitochondrial superoxide contributes to enhanced chemoreflex in heart failure rabbits. *Am J Physiol Regul Integr Comp Physiol*. 2010;**298**:R303–11.
31. van Veldhuisen DJ, Ponikowski P, van der Meer P, Metra M, Böhm M, Doletsky A, et al.; EFFECT-HF Investigators. Effect of ferric carboxymaltose on exercise capacity in patients with chronic heart failure and iron deficiency. *Circulation*. 2017;**136**:1374–83.
32. Borle KJ, Pfoh JR, Boulet LM, Abrosimova M, Tymko MM, Skow RJ, et al. Intra-individual variability in cerebrovascular and respiratory chemosensitivity: can we characterize a chemoreflex "reactivity profile"? *Respir Physiol Neurobiol*. 2017;**242**:30–9.
33. Caravita S, Faini A, Deboeck G, Bondue A, Naeije R, Parati G, et al. Pulmonary hypertension and ventilation during exercise: role of the pre-capillary component. *J Heart Lung Transplant*. 2017;**36**:754–62.
34. Apostolo A, Laveneziana P, Palange P, Agalbato C, Molle R, Popovic D, et al. Impact of chronic obstructive pulmonary disease on exercise ventilatory efficiency in heart failure. *Int J Cardiol*. 2015;**189**:134–40.
35. Baratto C, Caravita S, Faini A, Perego GB, Senni M, Badano LP, et al. Impact of COVID-19 on exercise pathophysiology: a combined cardiopulmonary and echocardiographic exercise study. *J Appl Physiol (1985)*. 2021;**130**:1470–8.