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Intravenous iron supplementation improves energy metabolism of exercising skeletal muscles without effect on either oxidative stress or inflammation in male patients with heart failure with reduced ejection fraction

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Abstract

Background: *Skeletal muscle dysfunction is a feature of heart failure (HF). Iron deficiency (ID) is prevalent in patients with HF associated with exercise intolerance and poor quality of life. Intravenous iron in iron deficient patients with HF has attenuated HF symptoms, however the pathomechanisms remain unclear. The aim of study was to assess whether intravenous iron supplementation as compared to placebo improves energy metabolism of skeletal muscles in patients with HF.*

Methods: *Men with heart failure with reduced ejection fraction (HFrEF) and ID were randomised in 1:1 ratio to either intravenous ferric carboxymaltose (IV FCM) or placebo. In vivo reduction of lactates by exercising skeletal muscles of forearm was analyzed. A change in lactate production between week 0 and 24 was considered as a primary endpoint of the study.*

Results: *There were two study arms: the placebo and the IV FCM (12 and 11 male patients with HFrEF). At baseline, there were no differences between these two study arms. IV FCM therapy as compared to placebo reduced the exertional production of lactates in exercising skeletal muscles. These effects were accompanied by a significant increase in both serum ferritin and transferrin saturation in the IV FCM arm which was not demonstrated in the placebo arm.*

Conclusions: *Intravenous iron supplementation in iron deficient men with HFrEF improves the functioning of skeletal muscles via an improvement in energy metabolism in exercising skeletal muscles, limiting the contribution of anaerobic reactions generating adenosine triphosphate as reflected by a lower in vivo lactate production in exercising muscles in patients with repleted iron stores.* (Cardiol J 2024; 31, 2: 300–308)

Keywords: heart failure, iron deficiency, skeletal muscles, exercise capacity, energy metabolism, physical fitness

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Introduction

Skeletal muscle dysfunction constitutes an important pathophysiological feature of heart failure (HF) and contributes to debilitating symptomatology of this disease syndrome, i.e., impaired exercise capacity and increased perception of dyspnoea, also during submaximal exercises [1–3]. Most importantly, these abnormalities translate into poor quality of life and unfavourable clinical outcomes in patients with HF [4], and therefore constitute clinically relevant targets for novel therapies.

Iron deficiency (ID) has been demonstrated to be prevalent in patients with HF [5, 6], and to be associated with impaired exercise capacity in these patients [5, 7, 8]. Most importantly, intravenous (IV) iron supplementation in iron deficient patients with HF has attenuated HF symptoms, and markedly improved exercise capacity and health related quality of life [9–13] as well as reduced the risk of recurrent HF hospitalizations [9–13].

Intriguingly, despite overwhelming evidence on clinical efficacy of IV iron repletion in patients with HF, the pathomechanisms responsible for these advantageous effects remain unclear. Being that the micronutrient critically needed for intracellular energy generation [14–16], iron is considered to play an important role in energy metabolism both within myocardial and skeletal muscle tissue [14–16]. Experimental evidence indicates that depleted intracellular iron leads to inefficient aerobic processes with adaptive anaerobic reactions within skeletal muscle tissue, and as a consequence decreases work [15–18] capacity. Recently, it has been shown that IV iron repletion in patients with HF improves skeletal muscle energetics as reflected by a shorter phosphocreatine recovery half-time on phosphorus magnetic resonance spectroscopy [19].

This paper is a complementary mechanistic study which aimed to assess whether IV iron supplementation as compared to placebo improves energy metabolism of exercising skeletal muscles in iron deficient male patients with HF, along with its effects on skeletal muscle performance, inflammation and oxidative status.

Methods

Study design

Herein, is reported a single-center, randomised (1:1), double-blind placebo-controlled study.

Caucasian outpatients with stable heart failure, reduced ejection fraction (HFrEF) and concomitant ID were examined. There following inclusion criteria of the study are: (1) male sex and age >18 years, (2) a documented history of chronic HF (established diagnosis of chronic HF according to the criteria of the European Society of Cardiology [20]) of at least 6-month duration, (3) ID defined as serum ferritin < 100 ng/mL, or serum ferritin 100–299 ng/mL with transferrin saturation (TSAT) < 20% [10, 11], (4) hemoglobin level ≥ 10 g/dL and ≤ 15 g/dL, (5) left ventricular ejection fraction ≤ 40% as assessed in echocardiography examination, not older than 6 months at the time of randomization, (6) New York Heart Association class I–III, (7) clinical stability along with unchanged HFrEF pharmacotherapy for at least 1 month preceding randomization, (8) signed informed consent form. Exclusion criteria included: (1) acute coronary syndrome or an episode of acute HF within 3 months preceding the study, (2) the therapy for anemia (including the use of erythropoiesis-stimulating agents) or/and ID (either IV or oral iron therapy) within 12 months prior to the study, (3) chronic infectious disease or symptoms of acute infection at the time of randomization, (4) a history of autoimmune, hematological or malignant disease (cancer), (5) muscular or neuro-muscular disorders, (6) dementia or significant cognitive dysfunction, or (7) simultaneous participation in any clinical trial.

Between April 16, 2014 and January 28, 2016 patients were randomly assigned in a 1:1 ratio to receive either ferric carboxymaltose (FCM) (provided by Vifor Pharma, Glattbrugg, Switzerland) or placebo (normal saline).

The trial was conducted in strict compliance with Good Clinical Practice from the International Council for Harmonisation (ICH GCP) and with the Declaration of Helsinki, and its protocol was approved by the local ethics committee (Bioethics Committee, Wroclaw Medical University, Consent No. 218/2014). All subjects gave written informed consent, before any trial-related procedure was performed.

Study procedures and visit schedule

The study intervention was an IV administration of FCM. Medication was administered as 10 or 20 mL of FCM (which is an equivalent of 500 or 1000 mg of iron, respectively) diluted in normal saline (0.9% weight/volume NaCl) to 100 mL volume. IV drop infusion was administered over at least 15 minutes. Normal saline was administered as placebo as per the instructions for active therapy. FCM dose was determined by the subject's body weight and hemoglobin value. The first dose was administered for all randomized subjects at week

0 after performing all planned procedures. The subsequent doses were administered as a part of the outpatient visits at week 6 and week 12 based on the following dosing scheme. At week 0, patients with hemoglobin ≥ 10 g/dL and ≤ 14 g/dL received 1000 mg of FCM, whereas those with hemoglobin > 14 g/dL and ≤ 15 g/dL were given 500 mg of FCM (regardless of body weight). At week 6, the study treatment was administered only to those with body weight ≥ 70 kg and hemoglobin ≥ 10 g/dL and ≤ 14 g/dL (500 mg of FCM). Additional doses of FCM were applied at week 12 for subjects in whom ID persisted, and for whom hemoglobin was ≥ 10 g/dL and ≤ 15 g/dL at those visits (500 mg of FCM, regardless of body weight).

Because FCM is a dark-brown solution that is easily distinguishable from the saline placebo, study personnel responsible for the preparation and administration of the study drug were aware of the group assignments (remained unblinded) and therefore were not involved in any study assessments. The laboratory results regarding iron status and hemoglobin were available only to the unblinded study personnel. To ensure that patients were unaware of the study treatment they were receiving, black syringes were used to administer the study drug and a curtain (or something similar) was used to shield the injection site from the patient's view, as in previous clinical trials with FCM [10, 11].

Study assessments and endpoints

The following parameters were assessed at baseline (week 0) and at the end of follow-up (week 24) (details on methodology are provided below):

- Energetic performance of exercising skeletal muscles:
  - production of lactates by exercising skeletal muscles of forearm (a difference in lactate level before and after exposure to standardised forearm exercise in peripheral blood derived from exercising muscles) (a change in lactate production between week 0 and 24 was considered as a primary endpoint of a study);
- Functional performance of skeletal muscles and global exercise capacity:
  - quadriceps strength;
  - six-minute walking test distance;
- Biomarkers related with functioning of skeletal muscles:
  - serum levels of irisin and hemojuvelin;
- Iron and anemic status assessed in peripheral blood:
  - serum ferritin, TSAT, the presence of ID,
  - hemoglobin level;
- Inflammatory status:
  - serum levels of C-reactive protein (CRP), tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta, IL-6, IL-22;
- Oxidative status:
  - serum activity of glutathione S-transferase (GST), catalase, superoxide dismutase (SOD).

Methodology

Energetic performance of exercising skeletal muscles. It was expressed as a production of lactates by exercising skeletal muscles of the forearm (a difference in lactate level before and after exposure to standardised forearm exercise in peripheral blood derived from exercising muscles, and expressed in mOsm/L.

The handgrip (forearm flexors compartment) exercise session performed by the dominant (mainly right) arm was considered as a standardised exercise load provided to this area of skeletal muscles. Briefly, the patient was requested to rhythmically handgrip the electronic dynamometer for 300 s at 50% of predetermined maximal voluntary contraction (MVC) (150 squeezes per exercise). The frequency of squeezing was driven by an electronic metronome and the real-time % of MVC curve was displayed on a large LCD monitor to help the patient to precisely follow the required grip strength and the exercise pace. Immediately after the exercise the patient performed an additional assessment of MVC to compare with its baseline (pre-exercise) value. Furthermore, before the exercise a superficial vein was catheterized (retrograde direction) in the antecubital fossa to obtain muscular blood metabolic parameters before and immediately after the handgrip exercise (standardised analysis of venous blood gases was performed to assess lactate level). Antecubital fossa veins are connected to deep veins of the forearm draining forearm flexors and metabolites detected in this area reflect, in vivo, the metabolism of exercising forearm muscles [21].

Quadricep strength. Peak quadricep torque (Nm) was measured in both lower extremities during a maximal dynamic and isometric knee extension with the hip in a 90° flexion while a patient sitting in a rehabilitation armchair type UPR 1A (Summer, Opole, Poland). Measurements were repeated at least 3 times and the highest value was recorded. Quadriceps muscle strength (N, a maximal dynamic and isometric strength) was calculated as a peak quadricep torque (Nm) and was divided

by a distance between a rotation axis and a point where lifted weights were attached (m).

Six-minute walking test. The six-minute walk test was performed in a long, straight hospital corridor, over a 30-m distance [22, 23]. Each participant was asked to walk (not run) back and forth along the corridor as briskly as possible, so that the longest possible distance was covered in 6 minutes. The participant was allowed to slow down or stop and rest, if necessary, particularly in the case of symptoms such as severe dyspnoea or fatigue. During any rest period, the participant was informed of the elapsed time and encouraged to recommence walking when the symptoms attenuated enough to allow walking. However, the test was discontinued if the symptoms persisted. The participant was also allowed to discontinue the test at will at any time. Moreover, the test was interrupted by the investigator immediately one of the following symptoms appeared: chest pain that did not resolve at rest, dyspnoea precluding continuation of walking, cramps of the lower limb muscles, balance difficulty, severe sweating, pallor, or cyanosis. Otherwise, every 2 minutes during the test, an investigator informed the participant of the amount of time left and encouraged him to continue the test. At 6 minutes, the participant was advised to stop and be seated. An investigator immediately measured post-exercise arterial blood pressure and pulse rate. The participant assessed subjective fatigue and dyspnoea levels with the modified Borg scale from 0 (none) to 10 (maximal). The distance walked was measured to the nearest whole meter.

Measurements performed in peripheral blood. In all participants venous blood samples were taken in the morning following an overnight fast. Hematological measurements were made in fresh venous blood with EDTA.

Parameters related with inflammatory status and those reflecting the functioning of skeletal muscles. The following particles were measured in either plasma or serum using a commercially available enzyme-linked immunosorbent assays (ELISA): hemojuvelin (ng/mL) (Cloud-Clone Corp., CCC, Wuhan); irisin (μg/mL) (BioVendor, Brno, Czech Republic); IL-6 (pg/mL) (R&D Systems, Minneapolis, MN, USA); IL-22 (pg/mL) (R&D Systems); IL-1β (pg/mL) (R&D Systems); TNF-α (pg/mL) (R&D Systems). The dilution of a series of serum/plasma samples were standardised in order to obtain absorption values at the middle of the standard curve. All measurements were performed in triplicate. Optical density at 450 nm was measured, with a reading time of 1 s, using a microtiter plate reader (BioTek, Synergy HTX).

Parameters reflecting oxidative stress. Activities of the following antioxidant enzymes were established using commercially available kits from Cayman Chemical Company (Ann Arbor, Michigan, USA): GST (nmol/min/mL), SOD (U/mL). All measurements were performed in triplicate according to manufacturer instructions.

Parameters related with iron and anaemic status. Hemoglobin concentration was measured using the ADVIA 2120 system (Siemens). Anemia was defined according to World Health Organization as hemoglobin concentration < 12 g/dL in women and < 13 g/dL in men. The following blood biomarkers reflecting iron metabolism were measured directly: serum ferritin (μg/L), iron (mg/dL), and total iron binding capacity (TIBC, mg/dL). TSAT was calculated as the ratio of serum iron (mg/dL) and TIBC (mg/dL) multiplied by 100 and expressed as a percentage. Serum ferritin was measured using an immunoassay based on electrochemiluminescence with the Elecsys 2010 system (Roche). Serum iron and TIBC were assessed using a substrate method with the Konelab Prime 60i system (Thermo Scientific). ID was defined as serum ferritin level < 100 μg/L or serum ferritin 100–299 μg/L in combination with a TSAT < 20% [10, 11].

Other laboratory measures. Serum level of high-sensitivity CRP (hs-CRP, mg/L) was assessed using immunonephelometry with BN II System (Siemens). Plasma level of N-terminal pro-B type natriuretic peptide (pg/mL) was measured using an immunoassay based on chemiluminescence with Dimension RxL system (Siemens). Estimated glomerular filtration rate (mL/min/1.73 m^2) was calculated using the Modification of Diet in Renal Disease equation [24].

All randomized patients were followed for the occurrence of prespecified outcomes throughout the follow-up period, regardless of whether the study participants were taking their study treatment or were compliant with study procedures. Throughout the follow-up period, all appropriate treatments for HF or other medical conditions could be initiated, altered or halted at the clinical discretion of the healthcare provider according to each patient's individual indications or contraindications.

Statistical analyses

Most continuous variables had a normal distribution, and were expressed as a mean ± the

standard error of the mean. Categorized variables were expressed as a number and percentage. The intergroup differences were tested using the Mann-Whitney U-test for unpaired samples.

The efficacy analyses were performed on the full-analysis set in accordance with the intention-to-treat principle. Treatment effect analysis was an unpaired comparison of the changes in continuous variables between the treatment arms using the Student t-test. Summary statistics include the point estimates of week 0 and 24 for continues variables, the change from baseline to week 24, and the estimates and 2-sided 95% confidence intervals for the difference between two study treatment arms.

P-value of < 0.05 was considered statistically significant. Statistical analyses were performed using the STATISTICA 13.1 data analysis software system (StatSoft).

Results

Twelve and 11 iron deficient male patients with HFrEF to a placebo arm and a IV FCM arm, were recruited, respectively. At baseline, there were no differences in clinical variables, comorbidities and applied treatment between these two study arms (Table 1). Intravenous FCM therapy as compared to placebo reduced the exertional production of lactates in exercising skeletal muscles (a change in lactates at week 0 and 24 in a placebo arm: 3.8 ± ± 0.2 and 2.5 ± 0.2, a change in lactates at week 0 and 24 in a IV FCM arm: 3.6 ± 0.3 and 1.6 ± 0.3; $p < 0.05$ for a difference between these study arms), which was associated with a numerical increase in quadricep strength and six-minute walking distance (Table 2, Fig. 1). These effects were accompanied by a significant increase in both serum ferritin and TSAT in the IV FCM arm (serum ferritin at week 0 and 24: 83 ± 18 and 325 ± 66; $p < 0.001$, TSAT at week 0 and 24: 20.6 ± 1.8 and 28.3 ± 1.6; $p < 0.05$), which was not demonstrated in the placebo arm (serum ferritin at week 0 and 24: 85 ± 14 and 96 ± 21; $p > 0.05$, TSAT at week 0 and 24: 19.0 ± ± 2.1 and 19.2 ± 2.5; $p > 0.05$). There were no changes in hemoglobin, biomarkers reflecting either functioning of skeletal muscles (irisin, hemojuvelin), inflammation (CRP, TNF-α, IL-β, IL-6, IL-22) or oxidative stress (GST, catalase, SOD) in both study arms throughout the study (Table 2).

Table 1. Baseline characteristics, comorbidities, and heart failure treatment among men with heart failure with reduced ejection fraction included in two study arms: placebo vs. intravenous ferric carboxymaltose.

	Placebo (n = 12)	IV FCM (n = 11)
Demographics and clinical measures		
Age [years], mean ± SEM	68 ± 3	63 ± 5
Male gender	12 (100%)	11 (100%)
Caucasian race	12 (100%)	11 (100%)
Body mass index [kg/m^2]	28.3 ± 1.3	28.1 ± 1.3
Ischemic etiology of HF	11 (92%)	9 (81%)
NYHA functional class		
II	8 (67%)	8 (73%)
III	4 (33%)	3 (27%)
LVEF [%]	30 ± 2	30 ± 2
Comorbidities		
Coronary artery disease	11 (92%)	9 (81%)
Paroxysmal atrial fibrillation	1 (8%)	2 (18%)
Diabetes mellitus	6 (50%)	3 (27%)
Dyslipidaemia	8 (67%)	6 (55%)
COPD	1 (8%)	0 (0%)
Peripheral artery disease	3 (25%)	2 (18%)
Arterial hypertension	5 (42%)	8 (73%)
Treatment		
ACEI or ARB	11 (92%)	11 (100%)
MRA	9 (75%)	8 (72%)
Beta-blocker	11 (92%)	11 (100%)
Digoxin	1 (8%)	0 (0%)
Ivabradine	2 (17%)	2 (18%)
Diuretic	5 (42%)	6 (54%)
Statin	12 (100%)	10 (91%)
ICD/CRT-D	11 (92%)	4 (36%)

Data is presented as mean plus/minus standard error of mean (SEM) for continues variables, numbers with % for categorized variables. IV FCM — intravenous ferric carboxymaltose; HF — heart failure; NYHA — New York Heart Association; LVEF — left ventricular ejection fraction; COPD — chronic obstructive pulmonary disease; ACEI — angiotensin converting enzyme inhibitors; ARB — angiotensin II antagonists; MRA — mineralocorticoid receptor antagonists; ICD/CRT-D — implantable cardioverter-defibrillator/ /cardiac resynchronization therapy with defibrillator function

Discussion

In the present study it was demonstrated that intravenous iron supplementation in iron deficient men with HFrEF improves energy metabolism in skeletal muscles. Intravenous therapy with FCM resulted in a significant increase in serum ferritin and TSAT, and the restoration of iron stores in the body was accompanied by a smaller in vivo production of lactates by exercising skeletal muscles, indicating the lower contribution of anaerobic processes generating adenosine triphosphate (ATP) in this tissue.

Table 2. The effect of intravenous ferric carboxymaltose vs. placebo on muscle energetics assessed in vivo, skeletal muscle functioning, and biomarkers related with functioning of skeletal muscles, iron status, inflammatory and oxidative status in men with heart failure with reduced ejection fraction.

	Baseline		End of study		Treatment effect (95% CI)
	Placebo (n = 12)	IV FCM (n = 11)	Placebo (n = 12)	IV FCM (n = 11)	
Energetic performance of exercising skeletal muscles					
Lactate [mOsm/L] baseline	1.4 ± 0.1	1.3 ± 0.2	1.6 ± 0.2	1.3 ± 0.1	0.1 (–0.3 to 0.4)
Lactate [mOsm/L] change after exercise	3.8 ± 0.2	3.6 ± 0.3	2.5 ± 0.2***	1.6 ± 0.3**	–0.8 (–1.6 to 0.0)*
Functional performance of skeletal muscles and global exercise capacity					
Quadriceps strength	87 ± 4	84 ± 4	89 ± 5	95 ± 6	7 (–1 to 15)
6MWT	463 ± 31	474 ± 15	475 ± 24	492 ± 14*	6 (–25 to 37)
Biomarkers related with functioning of skeletal muscles					
Hemojuvelin [ng/mL]	3.52 ± 0.10	3.36 ± 0.06	3.11 ± 0.08***	3.44 ± 0.29	0.41 (–0.23 to 1.05)
Irisin [ng/mL]	1.32 ± 0.16	1.58 ± 0.18	1.20 ± 0.12	1.01 ± 0.08*	–0.32 (–0.9 to 0.26)
Iron status and anaemic status assessed in peripheral blood					
Ferritin [ng/mL]	85 ± 14	83 ± 18	96 ± 21	325 ± 66***	231 (115 to 346)***
Ferritin < 100 ng/mL	8 (67)	9 (82)	8 (67)	0 (0)***	–
Transferrin saturation [%]	19.0 ± 2.1	20.6 ± 1.8	19.2 ± 2.5	28.3 ± 1.6 **	7.4 (1.8 to 13.1)*
Transferrin saturation < 20%	8 (67)	6 (55)	7 (58)	1 (9)	–
Iron deficiency [%]	12 (100)	11 (100)	11 (92)	3 (27)***	–
Hemoglobin [g/dL]	13.6 ± 0.3	13.6 ± 0.3	13.6 ± 0.3	13.8 ± 0.3	0.2 (–0.5 to 0.8)
Inflammatory status					
High sensitive CRP [mg/L]	3.4 ± 0.9	2.3 ± 0.7	3.3 ± 1.1	3.2 ± 0.9	1.0 (–1.2 to 3.2)
TNF-alfa [pg/mL]	0.50 ± 0.00	7.05 ± 5.12	1.07 ± 0.38	0.50 ± 0.00	–7.13 (–18.57 to 4.32)
IL-1beta [pg/mL]	0.10 ± 0.05	0.05 ± 0.00	0.07 ± 0.02	0.18 ± 0.13	0.16 (–0.15 to 0.47)
IL-6 [pg/mL]	5.62 ± 1.99	3.15 ± 0.94	3.70 ± 1.08	5.71 ± 1.88	3.43 (–2.16 to 9.02)
IL-22 [pg/mL]	52.75 ± 16.45	51.63 ± 7.38	48.92 ± 7.20	46.75 ± 4.33	–16.96 (–38.69 to 4.77)
Oxidative status					
GST [nmol/min/mL]	2.76 ± 037	2.05 ± 0.51	2.65 ± 0.26	2.76 ± 0.36	0.61 (–0.78 to 1.99)
Catalase [nmol/min/mL]	49.27 ± 6.69	45.64 ± 8.19	55.43 ± 6.10	40.38 ± 5.12	–12.01 (–39.08 to 15.06)
SOD [U/mL]	0.41 ± 0.07	0.29 ± 0.04	0.28 ± 0.06	0.23 ± 0.06	0.01 (–0.16 to 0.18)

Data is presented as mean ± standard error of mean for continues variables, numbers with percent for categorized variables; CI — confidence interval; IV FCM — intravenous ferric carboxymaltose; 6MWT — six-minute walk test; CRP — C-reactive protein; TNF — tumor necrosis factor; IL — interleukin; GST — glutathione S-transferase; SOD — superoxide dismutase; *p < 0.05; **p < 0.01; ***p < 0.001

There is enormous evidence demonstrating that IV iron supplementation improves quality of life and exercise capacity in patients with HF among all other approved symptomatic therapies [9, 10, 25]. Importantly, these effects seem to be independent on the effects of erythropoiesis [26, 27]. It is suggested that iron incorporated in iron-depleted myocardial and skeletal muscles and acting locally improves energy metabolism. There is substantial experimental evidence demonstrating, in vitro, that iron supplementation improves the function of cardiomyocytes and skeletal myocytes, and these effects are partially due to better function of mitochondria in these tissues [28–31].

The current study provides complementary evidence to data published by Charles-Edwards et al. [19]. The authors demonstrated that intravenous repletion of iron deficiency by iron isomaltoside enhanced skeletal muscle energetics in iron deficient patients with HF, as reflected by shorter PCr recovery half-times (PCr $t_{1/2}$) on phosphorus magnetic resonance spectroscopy [19]. In the present study another measure was applied allow-

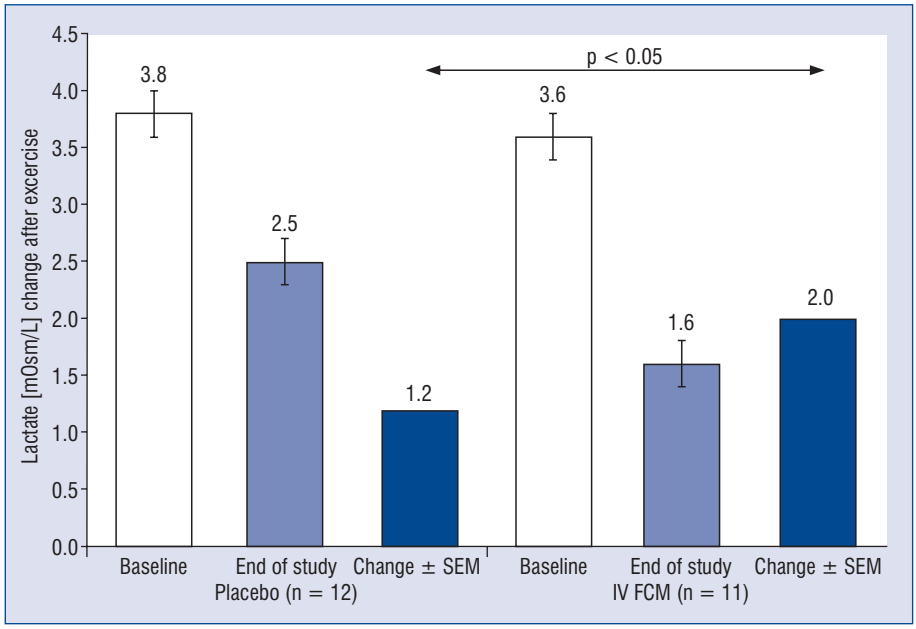

Figure 1. Reduction in exertional in vivo lactate production in skeletal muscles in men with heart failure with reduced ejection fraction due to intravenous iron therapy as compared to placebo; IV — intravenous; FCM — ferric carboxymaltose; SEM — standard error of mean.

ing assessment in vivo energetics of exercising skeletal muscles [21]. Lactates were measured in antecubital fossa veins that are connected to deep veins of the forearm draining forearm flexors and metabolites detected in this area reflect in vivo, the metabolism of exercising forearm muscles.

Limitations of the study

This was a relatively small mechanistic study including a small number of subjects in both study arms. Moreover, the study was limited only to men and patients with HFrEF.

Due to the small group of respondents, in order to maintain the homogeneity of the group, only men were included in the study.

Taking into account the influence of hormones on skeletal muscles the decision was dictated by the exclusion of hormonal variability among both sexes.

It was fully agreed that further studies in larger and more diverse populations are warranted to confirm and generalize these findings.

Nevertheless, the methodology applied used previously to investigate directly in vivo energetics of skeletal muscles in HF.

Conclusions

Intravenous iron supplementation in men with HFrEF improves the functioning of skeletal muscles via an improvement in energy metabolism in exercising skeletal muscles, limiting the contribution of anaerobic reactions generating ATP as reflected by a lower in vivo lactate production in exercising muscles in patients with repleted iron stores.

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