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Iron status, catabolic/anabolic balance, and skeletal muscle performance in men with heart failure with reduced ejection fraction

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Abstract

Background: *Metabolic derangements related to tissue energetics constitute an important pathophysiological feature of heart failure. We investigated whether iron deficiency and catabolic/anabolic imbalance contribute to decreased skeletal muscle performance in men with heart failure with reduced ejection fraction (HFrEF), and whether these pathologies are related to each other.*

Methods: *We comprehensively examined 23 men with stable HFrEF (median age [interquartile range]: 63 [59–66] years; left ventricular ejection fraction: 28 [25–35]%; New York Heart Association class I/II/III: 17/43/39%). We analyzed clinical characteristics, iron status, hormones, strength and fatigability of forearm flexors and quadriceps (surface electromyography), and exercise capacity (6-minute walking test).*

Results: *None of the patients had anemia whereas 8 were iron-deficient. Flexor carpi radialis fatigability correlated with lower reticulocyte hemoglobin content (CHR, $p < 0.05$), and there was a trend towards greater fatigability in patients with higher body mass index and lower serum ferritin (both $p < 0.1$). Flexor carpi ulnaris fatigability correlated with lower serum iron and CHR (both $p < 0.05$). Vastus medialis fatigability was related to lower free and bioavailable testosterone (FT and BT, respectively, both $p < 0.05$), and 6-minute walking test distance was shorter in patients with higher cortisol/FT and cortisol/BT ratio (both $p < 0.05$). Lower ferritin and transferrin saturation correlated with lower percentage of FT and BT. Men with HFrEF and iron deficiency had higher total testosterone, but lower percentage of FT and BT.*

Conclusions: *Iron deficiency correlates with lower bioactive testosterone in men with HFrEF. These two pathologies can both contribute to decreased skeletal muscle performance in such patients.* (Cardiol J 2021; 28, 3: 391–401)

Key words: heart failure, anabolic hormones, iron status, skeletal muscles, exercise capacity

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Introduction

Metabolic derangements associated with abnormal energy generation, utilization, and storage, and hormonal disorders promoting and modulating these processes, constitute an important pathophysiological feature of heart failure (HF) [1–7]. Disordered energy metabolism contributes to the progression of myocardial dysfunction and abnormalities seen in other tissues (such us skeletal muscles), and these processes promote each other in the mechanism of a vicious circle [1, 3, 8]. Being closely associated with abnormal tissue energetics, both iron deficiency (ID) and catabolic/anabolic imbalance negatively impact symptoms, exercise capacity, and outcomes in patients with HF [9, 10].

In this study we investigated whether metabolic derangements associated with abnormal mitochondrial energy metabolism, namely ID and catabolic/anabolic imbalance, contribute to decreased skeletal muscle performance in men with HF with reduced ejection fraction (HFrEF). Additionally, we evaluated whether these two pathologies (ID and hormonal abnormalities) are related to each other.

Methods

Patients

We decided to prospectively recruit only male patients due to the significant hormonal differences (influencing the functioning of skeletal muscles) in men and women. Study participants were recruited among male patients of a tertiary referral cardiology department and related outpatient clinic dedicated to HF patients. In all participants we analyzed clinical characteristics and evaluated iron status, anabolic and catabolic hormones, skeletal muscle performance, and sub-maximal exercise capacity. Detailed inclusion and exclusion criteria are presented below.

Inclusion criteria were as follows:

- male sex, age > 18 years;
- left ventricular ejection fraction (LVEF) ≤ 40% as assessed in latest echocardiography;
- an established diagnosis of HF (according to the criteria of the European Society of Cardiology [11]);
- clinical stability with no hospitalizations (either planned or unplanned) within the last 30 days;
- written informed consent for participation in the study.

Exclusion criteria were as follows:

- acute coronary syndrome, coronary revascularization, or major surgery within 90 days preceding the study;
- malignancy (cancer) diagnosed within the previous 5 years;
- cognitive impairment or inability to perform all procedures related to the study;
- current or previous therapy with erythropoiesis-stimulating agents, intravenous iron, or hormonal therapy (except for finasteride administered for benign prostatic hyperplasia);
- muscular, neurological, or orthopedic disorders impairing muscle performance and/or physical fitness.

The protocol was approved by the Bioethics Committee of Wroclaw Medical University, and all subjects gave written informed consent for participation in the study. The study was conducted in accordance with the Helsinki Declaration.

Hematology, iron status, hormonal measurements, and other laboratory tests

In all participants venous blood samples were taken in the morning (8–10 a.m. — important for credible hormonal measurements) following an overnight fast. The majority of laboratory tests were made from fresh venous blood. Some parameters were measured from frozen serum/plasma (after centrifugation the cryotubes were stored at –70°C) after collecting the material for all study participants (at the end of the study). All laboratory tests were performed in one laboratory: the central hospital laboratory of the Military Hospital, Wroclaw, Poland.

Hematological measurements were made in fresh venous blood anticoagulated with ethylene diamine tetra-acetic acid. Hemoglobin concentration, red cell indices, and reticulocytes were measured using the ADVIA 2120 hematology system (Siemens). Anemia was defined according to the World Health Organization (WHO): hemoglobin concentration < 13 g/dL in men [12].

Serum ferritin was measured using an electrochemiluminescence immunoassay (ECLIA) with a Cobas e601 module (Roche Diagnostics). Serum iron and unsaturated iron binding capacity (UIBC) were assessed using the colorimetric method with the Konelab Prime 60i system (Thermo Scientific). Total iron-binding capacity (TIBC) was automatically calculated using serum iron and UIBC. Transferrin saturation (TSAT) was calculated as the ratio of serum iron (μg/dL) and TIBC (μg/dL) multiplied

by 100 and expressed as a percentage. ID was defined (according to HF guidelines) as serum ferritin < 100 μg/L or serum ferritin 100–299 μg/L in combination with TSAT < 20% [11]. Serum soluble transferrin receptor (sTfR, mg/L) was measured using immunonephelometry with the BN II System (Siemens). Additionally, the following parameters obtained from automated blood count (ADVIA 2120 hematology system) were considered indirect indices of iron status: reticulocyte hemoglobin content (CHR, pg) and the percentage of hypochromic red cells (PHRC, %) [9].

The plasma level of N-terminal pro-B type natriuretic peptide (NT-proBNP, pg/mL) was measured using a chemiluminescence immunoassay with the Dimension ExL system (Siemens). The serum level of high-sensitivity C-reactive protein (hs-CRP, mg/L) was assessed using immunonephelometry with the BN II System (Siemens). One missing hs-CRP value was imputed with an available non-hs-CRP value of 5.53 mg/L. The estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) was calculated using the Modification of Diet in Renal Disease equation [13].

For the assessment of catabolic/anabolic balance the following hormones were measured in morning venous blood: total testosterone (TT, nmol/L), estradiol (pg/mL), insulin-like growth factor-1 (IGF-1, ng/mL), and dehydroepiandrosterone sulfate (DHEAS, μg/dL). Based on albumin and sex hormone-binding globulin (SHBG) concentrations, we used an online calculator (http://www.issam.ch/freetesto.htm) to estimate the fraction of free testosterone (FT, this fraction has the most potent biological activity) and bioavailable testosterone (BT = FT + albumin-bound testosterone; BT fraction is available for peripheral tissues) [10, 14]. FT and BT were expressed in nmol/L and as the percentage of TT pool (%FT and %BT, respectively). We also measured morning cortisol (nmol/L), and the following ratios were calculated to evaluate the catabolic/anabolic balance in examined men with HFrEF: cortisol/TT, cortisol/BT, cortisol/FT, cortisol/IGF-1, and cortisol/DHEAS [5]. TT, estradiol, DHEAS, and SHBG were measured using ECLIA with a Cobas e411 module (Roche Diagnostics), and cortisol was measured with ECLIA using a Cobas e601 module (Roche Diagnostics). IGF-1 was measured using chemiluminescence immunoassay with a Liaison XL analyzer (DiaSorin).

Skeletal muscle strength and fatigability

For the assessment of skeletal muscle performance, we measured handgrip and quadriceps strength, and the fatigability of forearm flexors and the quadriceps. Handgrip strength (N) of a dominant upper extremity was measured using the electronic dynamometer (Noraxon), and after the training the average from three maximal voluntary contractions was used for further analyses. Right leg quadriceps strength was evaluated by measuring quadriceps torque using an armchair with an isometric dynamometer. The torque was measured in a sitting position with 90° flexion of the knee joint. The parameter was calculated for the maximal isometric knee extension maneuver. After the initial training, the measurements were repeated three times and the average value was used in further analyses.

Non-invasive surface electromyography (sEMG) was applied to objectively evaluate muscle fatigability in different muscle regions: forearm flexors (flexor carpi radialis and flexor carpi ulnaris) and quadriceps (vastus lateralis and vastus medialis) [15]. Rectus femoris muscle signal was not analyzed due to the overlapping myoelectric signal from the vastii [16]. For the purposes of current study, we used a four-channel sEMG station MyoTrace 400 (Noraxon) combined with a dedicated electronic handgrip dynamometer (or used with the aforementioned armchair to evaluate the quadriceps). The crude sEMG signal was processed using dedicated research software: MyoResearch XP (Noraxon). Briefly, during a 10-second isometric exercise at 50% of predetermined maximal handgrip/quadriceps contraction, the sEMG was recorded in four predefined regions, and after signal processing the decrease in frequency (of the total power range, Hz) between the first and the last second was calculated as an index of muscle fatigability (greater decrease in frequency indicates more tired muscle). Handgrip and quadriceps contraction curves in N and Nm, respectively, were displayed "live" on a large monitor to help the patient to precisely follow the required 50% of the maximum.

Sub-maximal exercise capacity

Standard 6-minute walking test (6MWT) was performed to assess sub-maximal exercise capacity. Patients were walking at a comfortable (self-set but as brisk as possible) pace along a marked 30 m hospital corridor to cover the longest possible distance during 6 minutes. In case of any significant symptoms (e.g. dyspnea), the patient was allowed to slow down or even stop and rest.

Statistical analyses

Continuous variables were expressed as a median with lower and upper quartile (interquar-

tile range). Categorized variables were expressed as a number and percentage. The intergroup differences between subjects with vs. without ID were tested using the Mann-Whitney U-test for unpaired samples or χ^2 test, where appropriate.

In the first part of the statistical analyses we investigated the relationships between muscle function and metabolic derangements. We calculated Spearman's rank correlation coefficients (r) to establish the relationships between HFrEF symptoms (New York Heart Association [NYHA] class), handgrip strength, quadriceps torque, indices of muscle fatigability, and 6MWT distance and the following: (1) clinical parameters (age, body mass index [BMI], LVEF, key laboratory parameters), (2) hematological parameters, (3) iron parameters, and (4) indices of catabolic/anabolic balance. Further, we calculated Spearman's rank correlation coefficients to investigate the relationships between iron and hormonal parameters.

Hormonal parameters in patients with vs. without concomitant ID as well as 6MWT distance according to NYHA class (I to III), hs-CRP (≥ 2 vs. < 2 mg/L), and cortisol/testosterone ratio (≥ vs. < median) were compared using the Kruskal-Wallis H test.

A p-value of < 0.05 was considered statistically significant. Statistical analyses were performed using STATISTICA 13.3 data analysis software (TIBCO Software).

Results

Baseline characteristics of the examined men with HFrEF

The baseline characteristics of the examined patients according to the presence of ID are presented in Table 1. Although none of patients was anemic according to WHO criteria, 8 patients were iron-deficient. All subjects were taking evidence-based HFrEF pharmacotherapy, and 22 of them had either an implantable cardioverter-defibrillator or cardiac resynchronization therapy.

Metabolic derangements, skeletal muscle performance, and exercise capacity

The relationships between clinical variables, iron status, hormonal parameters, skeletal muscle performance, and exercise capacity are presented in Table 2. In the examined men with HFrEF lower quadriceps strength correlated with higher sTfR and PHRC, but these associations were not valid for handgrip strength. Flexor carpi radialis fatigability was greater in patients with lower CHR, and there was a trend towards greater fatigability in subjects with higher BMI and lower serum ferritin. Analogously, flexor carpi ulnaris fatigability correlated with lower serum iron and lower CHR, and there was a trend towards greater fatigability with decreasing hemoglobin. Vastus medialis fatigability was inversely correlated with FT and BT. 6MWT distance was greater in patients with lower NYHA class as well as in those with lower hs-CRP, cortisol/BT ratio, and cortisol/FT ratio (Fig. 1, Table 2).

Iron status versus catabolic/anabolic balance in men with HFrEF

The associations between iron parameters and measured hormones are presented in Table 3. Serum ferritin was related to %FT, %BT, and estradiol, and TSAT correlated with %FT and %BT (all $p < 0.05$). Indirect measures of ID (PHRC and CHR) were not related to hormonal parameters. Although male patients with ID compared with those without ID had higher TT, both %FT and %BT were significantly lower in iron-deficient subjects (Fig. 2). SHBG was higher in men with HFrEF with vs. without ID (median 72 vs. 46 nmol/L, $p = 0.01$), but these two groups had comparable albumin concentrations ($p = 0.9$).

Discussion

The current study provides additional evidence that metabolic derangements related to disordered tissue energetics, namely ID and catabolic/anabolic imbalance, can contribute to decreased skeletal muscle performance in non-anemic men with stable HFrEF.

The complex and multifaceted skeletal and respiratory myopathy constitutes an important element of HF pathophysiology [17, 18], and muscle dysfunction contributes to the symptomatology of HF [8]. Importantly, the key role in limiting HF patients' sub-maximal and maximal exercise performance is attributed to increased skeletal muscle fatigability, which has already been demonstrated for HF as long as three decades ago [18–23]. There is evidence that early and extensive skeletal muscle fatigue in HF results from intrinsic pathology of this tissue rather than insufficient perfusion, decreased cardiac reserve, or abnormal neural signaling [19, 20, 23, 24]. Although skeletal myopathy constitutes an important pathophysiological feature of HF, the precise mechanisms underlying muscular changes are not fully understood. In our study we have demonstrated that ID and catabolic/

Table 1. Baseline characteristics of examined men (n = 23) with heart failure with reduced ejection fraction (HFrEF) according to the presence of iron deficiency.

Variables	All patients (n = 23)	Iron deficiency (+) (n = 8)	Iron deficiency (–) (n = 15)
Clinical parameters			
Age [years]	63 (59–66)	64 (60–66)	62 (56–65)
Body mass index [kg/m^2]	29.7 (27.2–34.7)	28.7 (25.9–29.7)	32.3 (27.2–35.2)
New York Heart Association class I/II/III	4/10/9 (17/43/39%)	1/3/4 (13/38/50%)	3/7/5 (20/47/33%)
Ischemic heart failure etiology	13 (56%)	5 (63%)	8 (53%)
Left ventricular ejection fraction [%]	28 (25–35)	27 (23–33)	30 (25–37)
High-sensitivity CRP[$] [mg/L]	1.59 (1.01–3.2)	1.43 (1.04–2.09)	1.73 (0.87–3.45)
Plasma NT-proBNP [pg/mL]	**1312 (454–2414)**	**2404 (1141–4764)**	**960 (257–1511)**[b]
eGFR [mL/min/1.73 m^2]	76 (59–93)	74 (57–83)	86 (69–93)
Hematological parameters and indices of iron status			
Hemoglobin [g/dL]	15.6 (14.1–16.1)	15.8 (14.8–16.5)	15.2 (14.1–16.1)
Reticulocytes [%]	7 (6–9)	8 (7–9)	7 (6–9)
Serum iron [μg/dL]	**101 (89–134)**	**93 (69–108)**	**126 (94–141)**[b]
Serum ferritin [μg/L]	**129 (96–336)**	**77 (55–98)**	**288 (129–383)**[d]
Serum soluble transferrin receptor [mg/L]	1.33 (1.09–1.84)	1.55 (1.36–2.05)	1.17 (0.95–1.54)[a]
Transferrin saturation [%]	**28 (20–35)**	**20 (18–28)**	**34 (28–37)**[c]
Reticulocyte hemoglobin content [pg]	33 (32–34)	33 (30–33)	33 (32–34)
Percentage of hypochromic red cells [%]	0.4 (0.2–0.9)	0.8 (0.4–1.6)	0.4 (0.1–0.6)
Hormones			
Total testosterone [nmol/L]	**18 (13–26)**	**26 (20–29)**	**16 (9–19)**[c]
Free testosterone [%]	**1.5 (1.3–1.9)**	**1.3 (1.1–1.4)**	**1.7 (1.5–2.1)**[b]
Bioavailable testosterone [%]	**37 (31–42)**	**32 (24–34)**	**40 (35–49)**[b]
Estradiol [pg/mL]	**24 (18–36)**	**38 (29–46)**	**21 (16–27)**[c]
Insulin-like growth factor 1 [ng/mL]	194 (158–212)	193 (190–207)	196 (157–213)
Dehydroepiandrosterone sulfate [μg/dL]	102 (72–149)	97 (64–211)	139 (72–149)
Cortisol [nmol/L]	388 (317–464)	402 (284–442)	388 (323–482)
Major comorbidities			
Arterial hypertension	15 (65%)	5 (63%)	10 (67%)
Chronic obstructive pulmonary disease	1 (4%)	1 (13%)	0 (0%)
Atrial fibrillation	15 (65)	6 (75%)	9 (60%)
Diabetes or prediabetes	10 (43%)	3 (38%)	7 (47%)
Skeletal muscle strength and sub-maximal exercise capacity			
Handgrip strength [N]	367 (334–399)	368 (337–402)	367 (334–399)
Right quadriceps torque [Nm]	84 (69–91)	87 (67–99)	79 (69–91)
6-minute walking test distance [m]	423 (395–495)	438 (401–520)	415 (385–495)

[$]One missing high-sensitivity (hs) CRP value was imputed with available non-hs-CRP value of 5.53 mg/L. CRP — C-reactive protein; NT-proBNP — N-terminal pro-B-type natriuretic peptide; eGFR — estimated glomerular filtration rate. Data are presented as median (with an interquartile range) or number (with percentage), where appropriate. Handgrip strength was measured for dominant upper extremity. Statistical significance legend for the comparisons between patients with vs. without iron deficiency: [a]$p < 0.1$ (trend), [b]$p < 0.05$, [c]$p < 0.01$, [d]$p < 0.001$. For details — see the 'Methods' section.

/anabolic imbalance can contribute to decreased skeletal muscle performance in men with HFrEF. It should be acknowledged that efficient energy metabolism of skeletal muscle tissue critically depends on the proper regulation of mitochondrial functioning, which is precisely orchestrated by undisturbed iron and hormonal status [25, 26]. Indeed, mammalian skeletal muscles are important

Table 2. The relationships between heart failure symptoms, skeletal muscle performance, sub-maximal exercise capacity, and iron status and catabolic/anabolic balance in men with heart failure with reduced ejection fraction.

Variables, units	NYHA class, 1 class	Handgrip strength[#] [N]	Flexor carpi radialis fatigability[$#] [Hz]	Flexor carpi ulnaris fatigability[$#] [Hz]	Quadriceps torque[#] [Nm]	Vastus lateralis fatigability[$#] [Hz]	Vastus medialis fatigability[$#] [Hz]	Six–minute walking test distance [m]
Clinical parameters								
Age [years]	–	–	–	–	–	–	–	–
Body mass index [kg/m^2]	–	–	0.36[a]	–	–	–	–	–
NYHA class, 1 class	–	–	–	0.38[a]	–	–	–	**–0.65[d]**
Left ventricular ejection fraction [%]	–0.35[a]	–	–	–	–	–	–	–
High-sensitivity CRP[$] [mg/L]	–	–	**–0.45[b]**	–	–	0.40[a]	–	**–0.50[b]**
Plasma NT–proBNP [pg/mL]	–	–	–	–	–	–	–	–
eGFR [mL/min/1.73 m^2]	–	–	–	–	–	–	–	–
Hematological parameters and indices of iron status								
Hemoglobin [g/dL]	–	–	–	–0.37[a]	–	–	–	–
Reticulocytes [%]	–	–	–	–	–	–	–	–
Serum iron [μg/dL]	–0.40[a]	–	–	**–0.47[b]**	–	–	–	–
Serum ferritin [μg/L]	–	–	–0.37[a]	–	–	–	–	–
Serum soluble transferrin receptor [mg/L]	–	–	–	–	**–0.42[b]**	–	–	–
Transferrin saturation [%]	–0.37[a]	–	–	–	–	–	–	–
Reticulocyte hemoglobin content [pg]	–0.39[a]	–	**–0.42[b]**	**–0.62[c]**	–	–	–	–
Percentage of hypochromic red cells [%]	–	–	–	–	**–0.53[b]**	–	–	–
Hormones and indices of catabolic/anabolic balance								
Total testosterone [nmol/L]	–	–	–	–	–	–	–	–
Free testosterone [nmol/L]	–	–	–	–	–	–	**–0.49[b]**	0.38[a]
Free testosterone [%]	–	0.36[a]	–	–	–	–	–	–
Bioavailable testosterone [nmol/L]	–	–	–	–	–	–	**–0.45[b]**	0.36[a]
Bioavailable testosterone [%]	–	–	–	–	–	–	–	–
Estradiol [pg/mL]	–	–	–	–	–	–	–	–
IGF-1 [ng/mL]	**0.43[b]**	–	–	–	–0.37[a]	–	–	–
DHEAS [μg/dL]	–	–	–	–	–	–	–	–
Cortisol [nmol/L]	–	–	–	–	–	–	–	–
Cortisol/IGF-1 ratio [nmol/μg]	–	–	–	–	–	–	–	–
Cortisol/total testosterone ratio	–	–	–	–	–	–	–	–
Cortisol/DHEAS ratio [nmol/10*μg]	–	–	–	–	–	–	–	–
Cortisol/bioavailable testosterone ratio	0.39[a]	–0.36[a]	–	–	–	–	–	**–0.45[b]**
Cortisol/free testosterone ratio	0.38[a]	–	–	–	–	–	–	**–0.44[b]**

Data are presented as Spearman's rank correlation coefficients (coefficients with p-value of > 0.1 are not presented). Statistical significance legend: [a]p < 0.1 (trend), [b]p < 0.05, [c]p < 0.01, [d]p < 0.001. NYHA — New York Heart Association; CRP — C-reactive protein; NT-proBNP — N-terminal pro-B-type natriuretic peptide; eGFR — estimated glomerular filtration rate; IGF-1 — insulin-like growth factor 1; DHEAS — dehydroepiandrosterone sulfate. [$]Muscle fatigability (in 4 different muscle regions) refers to the decrease in the frequency (of the total power range, Hz) of a processed electromyography signal between 1 and 10 second of an isometric exercise (greater decrease indicates more tired muscle region). [#]Dominant upper extremity and right lower extremity were tested. For details (including surface electromyography methodology) — see the 'Methods' section.

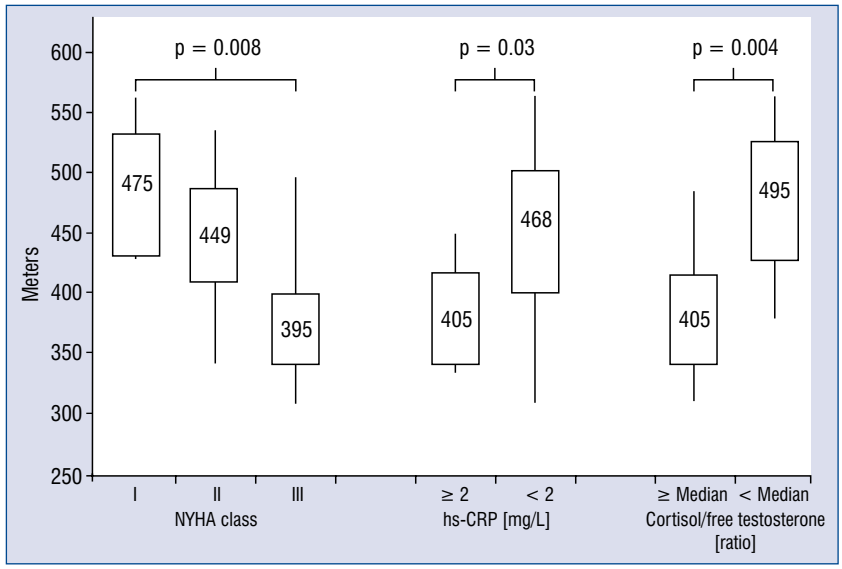

Figure 1. Six-minute walking test distance (box plots with median [number], interquartile range [box], and minimum/maximum [whiskers]) in men with heart failure with reduced ejection fraction according to New York Heart Association (NYHA) functional class, high-sensitivity C-reactive protein (hs-CRP), and the median of cortisol/free testosterone ratio (1478). P-values for the Kruskal-Wallis test are presented. For details — see the 'Methods' section.

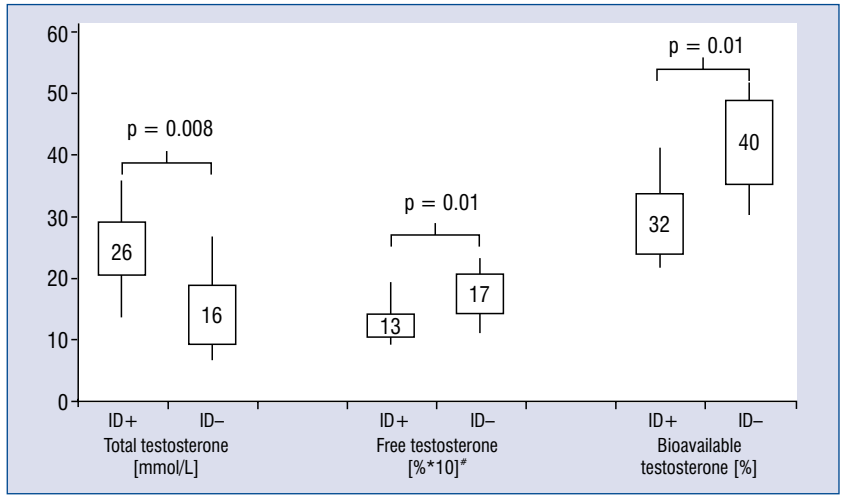

Figure 2. Total testosterone concentration with free and bioavailable testosterone fraction (box plots with median [number], interquartile range [box], and minimum/maximum [whiskers]) in examined men with heart failure with reduced ejection fraction according to the presence (ID+) or absence of iron deficiency (ID–). #Note that free testosterone values were multiplied by 10 to include this parameter in one figure with total and bioavailable testosterone (divide by 10 for normal values in percent). P-values for the Kruskal-Wallis test are presented. For details — see the 'Methods' section.

target tissues for circulating steroid hormones, in which they exert their direct anabolic properties [27, 28]. Undisturbed iron status is also necessary for the optimal functioning of mitochondria, and therefore it warrants cellular energy maintenance [29]. Importantly, iron determines tissue oxidative capacity, which is a major determinant of endurance and energetic efficacy during sub-maximal physical efforts [30].

Our results demonstrating the relationships between skeletal muscle performance and particular metabolic derangements are consistent

Table 3. The relationships between iron status, anabolic hormones, and measures of catabolic/anabolic balance in men with heart failure with reduced ejection fraction.

Variables [Units]	Hemoglobin [g/dL]	Reticulocytes [%]	Serum iron [μg/dL]	Serum ferritin [μg/L]	Soluble transferrin receptor [mg/L]	Transferrin saturation [%]	Reticulocyte hemoglobin content [pg]	Percentage of hypochromic red cells [%]
Total testosterone [nmol/L]	0.41[a]	–	–	–0.39[a]	–	–	–	–
Free testosterone [nmol/L]	0.41[a]	–	–	–	–	–	–	–
Free testosterone [%]	–	–	–	**0.49**[b]	–	**0.43**[b]	–	–
Bioavailable testosterone [nmol/L]	**0.49**[b]	–	–	–	–	–	–	–
Bioavailable testosterone [%]	–	–	–	**0.51**[b]	–	**0.45**[b]	–	–
Estradiol [pg/mL]	**0.47**[b]	–	–	**–0.47**[b]	0.36[a]	–	–	–
IGF-1 [ng/mL]	–	–	–	–	**0.48**[b]	–	–	0.41[a]
DHEAS [μg/dL]	–	–	–	–	–	–	–	–
Cortisol [nmol/L]	–	0.38[a]	–	–	–	–	–	–

Data are presented as Spearman's rank correlation coefficients (coefficients with p-value of > 0.1 are not presented). Statistical significance legend: [a]p < 0.1 (trend), [b]p < 0.05. IGF-1 — insulin-like growth factor 1; DHEAS — dehydroepiandrosterone sulfate. For details — see the 'Methods' section.

with previous studies conducted in this field. For example, Melenovsky et al. [31] demonstrated in an exercise phosphorus-31 magnetic resonance spectroscopy experiment that HF patients with ID had lower muscle strength, greater exertional muscle acidosis, and earlier metabolic shift to anaerobic metabolism. It is worth mentioning that we demonstrated in a previous study [32] that low serum ferritin correlates with inspiratory muscle weakness in men with HFrEF. Skeletal muscle dysfunction related to ID and catabolic/anabolic imbalance is a potential explanation why patients with ID or depleted anabolic drive have lower exercise capacity than subjects without these derangements [25, 26, 32–34]. It was previously demonstrated that intravenous iron therapy improves exercise capacity in patients with HFrEF and ID irrespective of anemia [35], and there is limited evidence that testosterone therapy may have similar beneficial effects [36]. In this context, the results of a small, randomized, double-blind, controlled study regarding iron isomaltoside in symptomatic HF should be acknowledged [37]. The authors demonstrated that intravenous iron repletion improves skeletal muscle energetics in both anemic and non-anemic subjects as assessed using phosphorus magnetic resonance spectroscopy [37]. Our study provides additional evidence regarding the consideration of HF as a "metabolic disease" [38, 39]. In our study, however, the distance covered in a 6MWT was related to catabolic/anabolic balance and inflammation, but the relationship with ID did not reach statistical significance. The latter was probably due to the relatively small number of examined patients. Importantly, we are able to partially compare clinical status, hemoglobin, and anabolic hormones of male patients from this study with our historical cohort of 205 men with stable, chronic HFrEF (LVEF ≤ 40%) recruited in 2001–2005 for another research project [33]. Although male HF patients from 2001–2005 had comparable age, NYHA class distribution, LVEF, NT-proBNP, and TT ($p > 0.05$ for all comparisons of mean ± standard deviation between the previous and this study), the current group of men with HFrEF had higher DHEAS (130 ± 98 vs. 88 ± 77 μg/dL, $p = 0.02$), IGF-1 (197 ± 52 vs. 134 ± 66 ng/mL, $p < 0.001$) and hemoglobin (15.3 ± 1.3 vs. 14.3 ± 1.5 g/dL, $p = 0.002$) as compared with the historical cohort [33]. The aforementioned data suggest that even clinically comparable groups of HF patients may subtly differ in particular hormonal parameters.

In this study we have also demonstrated the relationships between iron parameters and bioac-

tive testosterone. Although men with HFrEF and concomitant ID had higher TT compared with those without ID (and also higher SHBG, but not albumin), they presented with lower free and bioavailable fractions of this hormone. It should be acknowledged that the relationships between ID and hormonal status of men with HFrEF have not been studied so far, including large biomarker HF programs such as BIOSTAT-CHF [40]. The potential explanation of why depleted anabolic drive correlates with ID is related to impaired intestinal absorption and malnutrition [40]. There is clinical and experimental evidence that dysregulated catabolic/anabolic balance characterizing advanced HF promotes several maladaptive mechanisms within the gastrointestinal system, including intestinal hypoperfusion, edema, and anorexia [41–43]. The aforementioned pathomechanisms are responsible for disordered absorption of several microelements and further malnutrition, the pathologies of which are frequently observed in patients with HF [44, 45]. Decreased absorption of iron is considered one of the key mechanisms explaining how patients with HF develop ID, apart from accumulation of iron in the mononuclear phagocyte system [46]. Although the relationships between catabolic/anabolic balance and ID have not been studied in HF so far, we have some data on neuroendocrine signaling and iron status in this population. In one cross-sectional study regarding more than 700 patients with chronic HF, low TSAT was related to increased sympathetic activation, as reflected by higher circulating stress hormone norepinephrine [47]. Both increased sympathetic drive and catabolic/anabolic imbalance are involved in the complex pathomechanism of progressive catabolic state occurring in HF, and they both contribute to cardiac cachexia [48]. It remains unclear whether these unfavorable trajectories are further promoted or only accompanied by concomitant ID. It is worth noting that in experimental animals testosterone mediates systemic iron status through inhibition of the transcription of hepatic hepcidin — the key iron regulator [49, 50]. Further studies are required to determine independent effects of ID and catabolic/anabolic imbalance on skeletal muscle performance and exercise capacity in men with HFrEF.

Limitations of the study

We enrolled relatively a small number of subjects with HFrEF, and further studies in larger populations are needed not only to confirm the aforementioned relationships (metabolic derangements — skeletal muscle function; iron status — hormones), but also to evaluate independent effects of disordered iron homeostasis and catabolic/anabolic imbalance on skeletal muscle performance. Additionally, we examined only men with HFrEF, and there are no data presented regarding either female patients or subjects with the two remaining strata of LVEF (HF with preserved and mid-range ejection fraction). Finally, in the current study there was no control group, and the presented relationships should be re-evaluated in an age-matched group of healthy men without any cardiovascular disease.

Conclusions

In this preliminary study we have demonstrated that metabolic derangements related to energy generation and utilization, namely ID and catabolic/anabolic imbalance, can contribute to decreased skeletal muscle performance in men with HFrEF. Additionally, we have shown that there is a relationship between ID and reduced bioactive testosterone in these patients.

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