Circulation: Heart Failure

ORIGINAL ARTICLE

Skeletal Muscle Abnormalities and Iron Deficiency in Chronic Heart Failure

An Exercise ^{31}P Magnetic Resonance Spectroscopy Study of Calf Muscle

BACKGROUND: Heart failure (HF) is often associated with iron deficiency (ID). Skeletal muscle abnormalities are common in HF, but the potential role of ID in this phenomenon is unclear. In addition to hemopoiesis, iron is essential for muscle bioenergetics. We examined whether energetic abnormalities in skeletal muscle in HF are affected by ID and if they are responsive to intravenous iron.

METHODS AND RESULTS: Forty-four chronic HF subjects and 25 similar healthy volunteers underwent ^{31}P magnetic resonance spectroscopy of calf muscle at rest and during exercise (plantar flexions). Results were compared between HF subjects with or without ID. In 13 ID-HF subjects, examinations were repeated 1 month after intravenous ferric carboxymaltose administration (1000 mg). As compared with controls, HF subjects displayed lower resting high-energy phosphate content, lower exercise pH, and slower postexercise PCr recovery. Compared with non-ID HF, ID-HF subjects had lower muscle strength, larger PCr depletion, and more profound intracellular acidosis with exercise, consistent with an earlier metabolic shift to anaerobic glycolysis. The exercise-induced PCr drop strongly correlated with pH change in HF group (r=–0.71, P<0.001) but not in controls (r=0.13, P=0.61, interaction: P<0.0001). Short-term iron administration corrected the iron deficit but had no effect on muscle bioenergetics assessed 1 month later.

CONCLUSIONS: HF patients display skeletal muscle myopathy that is more severe in those with iron deficiency. The presence of ID is associated with greater acidosis with exercise, which may explain early muscle fatigue. Further study is warranted to identify the strategy to restore iron content in skeletal muscle.

Vojtech Melenovsky, MD, PhD*
Katerina Hlavata, MD*
Petr Sedivy, PhD
Monika Dezortova, PhD
Barry A. Borlaug, MD
Jiri Petrak, PhD
Josef Kautzner, MD, PhD
Milan Hajek, PhD, DSc

*Drs Melenovsky and Hlavata contributed equally to this work.

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WHAT IS NEW?

- Iron deficiency, common in heart failure, leads to muscle bioenergetic deficit and acidosis during exercise that is measurable by magnetic resonance spectroscopy.
- Iron-deficient heart failure subjects were more symptomatic, had lower peak muscle strength, larger energetic depletion, and more pronounced acidification of the muscle during exercise, consistent with metabolic shift towards anaerobic glycolysis.
- This study provides evidence that iron deficiency contributes to skeletal myopathy and impaired muscle function in chronic heart failure.

WHAT ARE THE CLINICAL IMPLICATIONS?

- Targeting iron deficiency can serve as a therapeutic target to improve exercise capacity in chronic heart failure patients, beyond the correction of hemoglobin levels.

Approximately half of patients with chronic heart failure (HF) have iron deficiency (ID) as a result of inadequate nutritional intake, impaired iron absorption or increased blood loss.[1,2] ID in HF patients is associated with greater symptom severity, worse exercise capacity, and increased mortality, independently of anemia.[2–4] In addition to its necessity for hemopoiesis, iron is an essential cofactor for multiple intracellular proteins that contain heme or iron-sulfur clusters, including complexes of the respiratory chain and myoglobin,[5–8] which are indispensable for normal function of cellular bioenergetic machinery.

In experimental animal models, severe ID itself, even in the absence of anemia or HF, causes skeletal myopathy,[9,10] although similar evidence in humans is less consistent.[10–12] Previous investigations established that patients with symptomatic HF often have impaired skeletal muscle performance[13–17]; but iron status was not quantified in those patients. It is unknown whether ID can worsen muscle bioenergetics or contribute to the HF-related myopathy in humans.[18] Because iron stimulates mitochondrial biogenesis and improves mitochondrial function,[19] the correction of muscle iron deficit could improve muscle bioenergetics[20] and enhance exercise performance.[21]

The aim of this study was to determine whether HF is associated with disruption of energetic metabolism in skeletal muscle at rest and during exercise, whether these abnormalities are affected by the presence of ID, and if any ostensible abnormalities might be corrected by systemic iron repletion. To address these questions, we performed rest and dynamic ^{31}P magnetic resonance spectroscopy (MRS), an established noninvasive technique to study muscle bioenergetics,[22,23] in healthy controls and in HF patients with or without ID, and repeated the examination in iron-deficient HF subjects after intravenous iron administration.

METHODS

Twenty-five healthy volunteers and 44 clinically stable subjects with chronic systolic HF of >6 months duration were recruited at the Institute for Clinical and Experimental Medicine (IKEM) in Prague. All subjects provided written informed consent, and this study was approved by the Ethics committee of the institution. HF subjects underwent blood sampling, physical examination, and Minnesota Living with HF Questionnaire assessment. B-type natriuretic peptide levels were measured (Abbott Architect, Chicago, IL). Routine clinical biochemistry was analyzed using automated Abbott Architect ci1600 analyzer (Abbott Laboratories). ID was defined as serum ferritin <100 μg·L^{-1} or ferritin 100 to 299 μg·L^{-1} and the transferrin saturation <20%.[24] The data that support the findings of this study are available from the corresponding author on reasonable request.

^{31}P MRS

All subjects underwent ^{31}P MRS of the gastrocnemius muscle (3T MR tomograph Siemens Trio,^{1}H/^{31}P surface dual coil, Rapid, Germany) at rest and during low-level isotonic exercise using custom-built MR-compatible ergometer, as described previously.[25] Maximal isometric strength of calf muscle was measured prior the study outside the MR machine using a compression pressure sensor, defined as the highest peak force achieved during 3 attempts in the supine position. During exercise, stroke length was continuously monitored via custom-build software. Work performed during exercise was calculated as the total stroke length of chosen weight. The gastrocnemius muscle area was calculated from transverse proton-density MR images using manual segmentation of images in program ImageJ (Figure 1A). Gastrocnemius muscle provides better signal/noise ratio while is still sensitive to changes of oxidative phosphorylation.[26] Resting acquisitions were performed by free induction decay sequence with the following parameters: repetition time 15 seconds, 16 acquisitions, sequence length: 4 minutes, flip angle: 90°. Exercise acquisitions with 6 minutes of plantar flexions each 2 seconds and fixed low-level workload of 7 kg (corresponding to 21±9% of maximal isometric strength) had the following parameters: repetition time 2 seconds, an acquisition of 420 measurements (60 spectra at rest, 180 during exercise, and 180 during recovery), total time: 14 minutes, flip angle: 42°.

MRS Signal Analysis

Quantification of the MR spectra was accomplished by jMRUI software using the AMARES time domain fitting routine as described previously.[25] After phasing the spectra, the peaks were fitted as single Lorentzians, except for the adenosine triphosphate (ATP) signals, where the γ- and α-ATP were fitted as Lorentzian doublets and β-ATP as a triplet. The β-ATP signal was used as a concentration reference.[23] From resting ^{31}P MR spectrum, relative

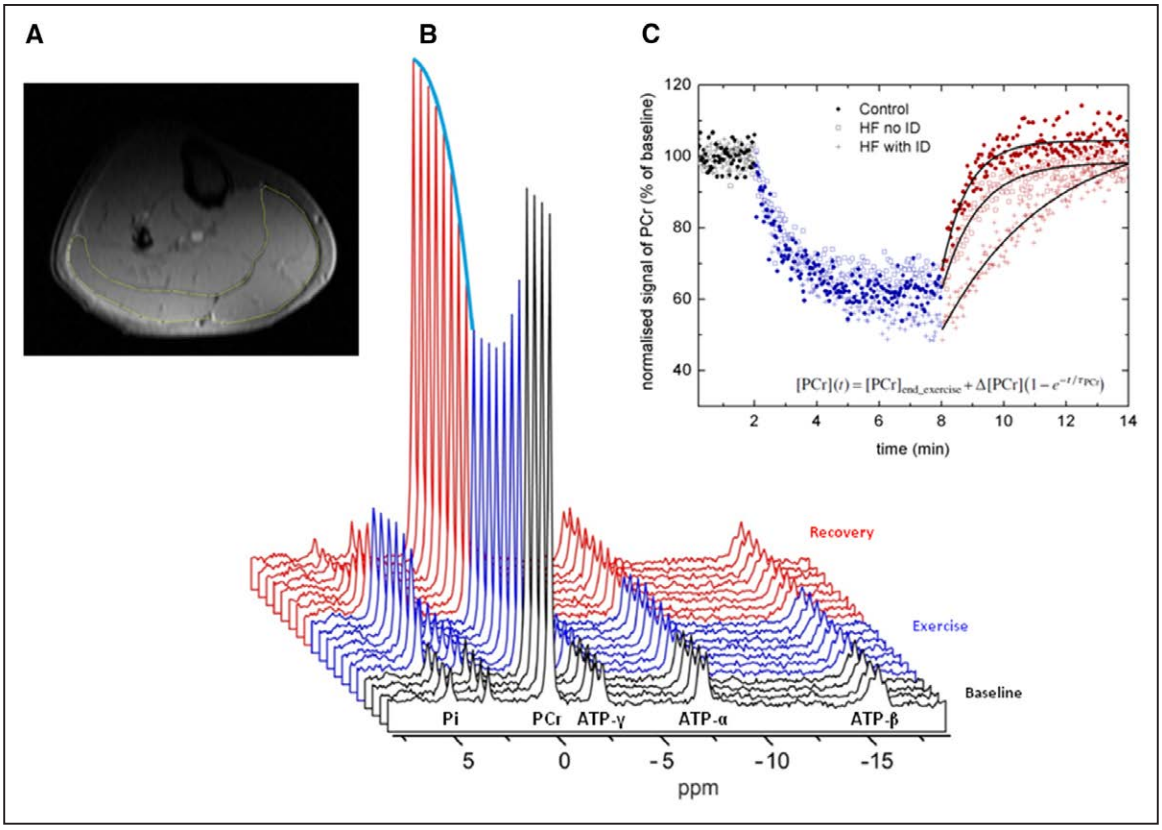

Figure 1. The principle of ^{31}P magnetic resonance spectroscopy (MRS) of calf muscle.
A, Orientation MR image of transversal section of calf muscle used for image acquisition. Gastrocnemius muscle is manually traced. **B**, Example of MRS spectra collected during baseline rest (black), during plantar flections exercise (blue), and during postexercise recovery (red). Note constant size of adenosine triphosphate (ATP-α,-β,-γ) peaks, note prompt reduction of phosphocreatine (PCr) peak and increased of inorganic phosphate (Pi) peak during exercise. **C**, Postexercise recovery is characterized by instant restoration of PCr concentration. The time constant of PCr recovery (τ_{PCr}) derived by curve fitting (see the equation), characterizes muscle oxidative phosphorylation in controls (solid dots) and heart failure (HF) patients with (crosses) and without (squares) iron deficiency (ID).

concentrations of phosphocreatine (PCr), ATP, and inorganic phosphate (Pi) were determined in relation to the total phosphorus signal intensity (Figure 1B and 1C). The chemical shift of Pi relative to PCr was used to calculate the intracellular pH according to the Henderson-Hasselbalch equation.[27]

From exercise datasets, the exercise-induced drop of PCr (ΔPCr in % of rest) and end-exercise pH was calculated. The kinetics of restoration of PCr concentration in the recovery period reflects de novo ATP synthesis. The time course of PCr concentration in recovery was fitted to a monoexponential function characterized by τ_{PCr} (time constant of PCr recovery). The initial rate of PCr recovery (V_{PCr}), representing the ATP turnover, was calculated as $\Delta PCr/\tau_{PCr}$. Absolute ADP concentration at the end of exercise was calculated according to the method of Kemp.[28] The maximal oxidative flux (Q_{max}), a measure of mitochondrial capacity, was calculated according to the Michaelis-Menten equation, taking into account free cytosolic ADP at the end of exercise.[22,25] The details of the method and rest-retest repeatability in our MR facility were recently described in detail.[25]

Iron Intervention Protocol

In 13 consecutive subjects with HF and ID, who fulfilled indications for intravenous iron therapy (FAIR-HF trial [Ferric Carboxymaltose in Patients With Heart Failure and Iron Deficiency] criteria)[24] and were willing to undergo MRS examination again, the study was repeated 1 month after intravenous administration of 2 doses of 500 mg intravenous ferric carboxymaltose (FCM, Ferinject 500 mg, Vifor, France). The first 500 mg FCM intravenous dose was applied immediately after the first visit, the second dose 2 weeks later. FCM is a stable polynuclear iron (III) hydroxide carbohydrate complex that prevents the partial release of free iron, allowing intravenous administration in high doses, because this iron is available only via reticuloendothelial processing. Short and long-term safety of intravenous FCM administration are well established, even in population with high cardiovascular risk.[29] We chose a higher FCM dose (but still within recommended limits[30]) but shorter course of therapy than in FAIR-HF trial[24] because of ethical reasons. Most of the patients were candidates for primary preventive implantable cardioverter-defibrillator, and we did not want to defer implantable cardioverter-defibrillator implantation for the sake of extended follow-up MR study. We reasoned that biological effects of iron would be detectable after 1 month, since red cell uptake of radio-labeled iron reaches >90% 24 days after single FCM dose.[30] All examinations were again obtained at follow-up, including blood tests, clinical examination, and questionnaire. The medication was kept the same between the first and the second examinations.

Data were analyzed using JMP12.2 software. Normality of distribution was checked with the Shapiro-Wilk test. Groups were compared using paired or nonpaired Student's *t* test for continuous, normally distributed data or using Wilcoxon and Wilcoxon

signed-rank test for non-normally distributed data, if not stated otherwise. Proportions were tested with χ^2 test. Correlations were tested using Pearson correlation coefficient. Linear univariate least squares regression model was used to identify determinants of muscle bioenergetic parameters. Where indicated, the interaction between variables and groups was tested by entering an interaction term into the model. Values are reported as arithmetic means±SD or medians (interquartile range), if not stated otherwise. *P* value <0.05 was considered significant.

RESULTS

The HF group consisted of predominantly middle-aged males with history of severely symptomatic HF (59% New York Heart Association III-IV) with reduced ejection fraction, mostly of nonischemic cause (Table 1). All HF subjects were treated with loop diuretics (mean furosemide dose 95 mg/d), 77% were on β-blockers, and 68% were on an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker. Controls were similar to HF group in age, sex, and body mass index. Seventy-two percent of subjects with HF had ID, and 36% had anemia (Table 1).

Muscle Bioenergetics in HF Compared With Controls

Despite similar body mass, gastrocnemius muscle area was smaller in HF compared with controls, and peak isometric force was reduced, consistent with HF-related skeletal muscle atrophy (Table 2). Concentrations of high-energy phosphates were significantly reduced, and Pi was increased in HF subjects compared with controls, though these differences were attenuated after normalization to muscle area. The ratios of ATP/Pi or PCr/Pi were reduced in HF, consistent with impaired resting energetic charge. During dynamic ^{31}P MR examination, subjects with HF performed less work, but PCr concentration dropped similarly to controls, so that PCr change per work unit was larger in HF than in controls (13±11 versus 6.4±3.1; *P*=0.003). Despite similar objective workload achieved, postexercise pH was lower in HF subjects compared with controls (Figure 2). The exercise-induced drop of PCr correlated with change of pH in HF group (*r*=–0.71; *P*<0.001) but not in controls (*r*=0.13, *P*=0.61; Figure 3). In the recovery phase, HF subjects displayed a longer PCr recovery time constant (τ_{PCr}), indicating slower replenishment of high-energy phosphates by oxidative phosphorylation after exercise.

The determinants of PCr kinetics in the recovery phase in patients with HF are summarized in Table 3 and Figure 4. Time constant of PCr recovery (τ_{PCr}) was inversely proportional to maximal mitochondrial activity (Q_{max}). Q_{max} demonstrated a strong inverse relation with age and weaker relation to body mass index and to presence of diabetes mellitus. The initial rate of PCr recovery (V_{PCr}) was also related to age. Maximal isometric strength was unre-

Table 1. Baseline Characteristics of HF Subjects and Controls

	Controls (n=25)	HF (n=44)	*P* Value	HF, No ID (n=12)	HF With ID (n=32)	*P* Value
Age, y	49±15	55±16	0.11	56±13	55±17	0.85
Male sex, %	60	75	0.19	83	72	0.43
BMI, kg·m^{-2}	28±4	27±6	0.83	29±8	27±5	0.28
NYHA class	…	2.7±0.7	…	2.8±1.0	2.6±0.7	0.68
Non-CAD HF cause, %	…	68	…	67	68	0.89
LVEF, %	…	27±10	…	29±11	26±9.1	0.48
HF duration, y	…	5.8±7.0	…	7.0±7.9	5.3±6.8	0.51
HF hospitalizations, n	…	1.5±1.5	…	1.2±1.3	1.7±1.6	0.27
MLHFQ sum score	…	34±22	…	30±18	36±23	0.37
BNP,* ng·L^{-1} *	…	407 (188–1399)	…	161 (103–179)	543 (253–1592)	0.007
Creatinine, mg·dL^{-1}	1.0±0.2	1.4±0.4	<0.001	1.4±0.3	1.4±0.4	0.71
Iron metabolism						
Hemoglobin, g·L^{-1}	145±14	134±18	0.01	146±13	129±17	0.002
Mean cell volume, fL	89±4	87±5	0.13	89±4	86±5	0.07
Ferritin,* µg·L^{-1}	…	90 (47–187)	…	239 (167–297)	70 (39–94)	0.001
Transferrin sat., %	…	21±13	…	31±9	17±13	0.0004
Transferrin, g·L^{-1}	…	3.1±0.7	…	2.6±0.4	3.2±0.7	0.001
Serum iron, µg·dL^{-1}	…	84±43	…	112±25	75±44	0.001

Groups are compared by unpaired *t* test, χ^2 test, or Wilcoxon test where appropriate. BMI indicates body mass index; BNP, B-type natriuretic peptide; CAD, coronary artery disease; HF, heart failure; ID, iron deficiency; IQR, interquartile range; LVEF, left ventricular ejection fraction; MLHFQ, Minnesota Living With Heart Failure Questionnaire; and NYHA, New York Heart Association.

*Values are medians (IQR); all other are means±SD.

Table 2. Muscle Characteristics and Bioenergetics in HF and Controls at Rest and Exercise

	Controls (n=25)	HF (n=44)	*P* Value	HF, no ID (n=12)	HF With ID (n=32)	*P* Value
Calf muscle characteristics						
Muscle area, cm^2	24±5	19±5	0.0002	21±6	19±5	0.36
Peak isometric force, N	453±151	356±143	0.02	439±125	325±139	0.03
Resting bioenergetics						
PCr	0.51±0.02	0.50±0.04	0.09	0.51±0.04	0.50±0.03	0.84
Pi	0.06±0.01	0.07±0.01	0.005	0.07±0.02	0.08±0.01	0.09
ATP	0.09±0.01	0.08±0.01	0.0006	0.08±0.01	0.08±0.01	0.74
PCr/Pi	8.2±1.5	7.1±1.8	0.01	8.1±2.3	6.8±1.4	0.09
ATP/Pi	1.5±0.3	1.2±0.4	0.001	1.4±0.5	1.1±0.3	0.14
pH rest	7.02±0.03	7.04±0.03	0.18	7.04±0.03	7.03±0.02	0.61
Exercise bioenergetics						
Work,* W	3.4 (3.1–4.7)	2.5 (1.6–3.6)	0.004	2.8 (1.9–3.6)	2.5 (1.6–3.9)	0.75
PCr drop, %	24±13	28±16	0.24	19±8	31±17	0.01
PCr drop/work,* W^{-1}	5.6 (4.3–8.1)	9.1 (4.6–19.8)	0.03	6.2 (3.6–18.3)	9.4 (5.0–19.7)	0.27
pH end	7.02±0.03	6.97±0.15	0.05	7.04±0.06	6.95±0.17	0.03
ADP end, $\mu mol \cdot L^{-1}$	27±9	30±9	0.23	24±7	32±9	0.01
Recovery bioenergetics						
τ_{PCr},* ms	41 (34–48)	55 (38–74)	0.01	45 (36–65)	59 (38–83)	0.32
V_{PCr},* $mmol \cdot s^{-1}$	0.22 (0.16–0.38)	0.23 (0.17–0.28)	0.68	0.18 (0.16–0.24)	0.24 (0.17–0.31)	0.18
Q_{max},* $mmol \cdot s^{-1}$	0.51 (0.38–0.77)	0.47 (0.36–0.56)	0.22	0.47 (0.40–0.49)	0.47 (0.32–0.63)	0.79

PCr, Pi, and ATP are expressed in relative units (fraction of the total spectral intensity), ADP in absolute units. Groups are compared by unpaired *t* test or Wilcoxon test where appropriate. ADP indicates adenosine diphosphate; ATP, adenosine triphosphate; HF, heart failure; ID, iron deficiency; PCr, phosphocreatine; Pi, inorganic phosphate; Q_{max}, maximal oxidative flux; τ_{PCr}, time constant of PCr recovery; and V_{PCr}, initial rate of PCr recovery.

*Values are medians (IQR); all other are means±SD.

lated to bioenergetics parameters but correlated with both hemoglobin and ferritin in HF group (both r=0.51, P=0.002).

Muscle Bioenergetics in HF With or Without Iron Deficit

HF subjects with ID had higher B-type natriuretic peptide levels, lower hemoglobin concentration (Table 1), and similar muscle mass, but lower isometric force per muscle area as compared with non-ID HF subjects (18±7 versus 22±6 $N \cdot cm^{-2}$, P=0.05). Iron status was not associated with alterations of resting muscle bioenergetics (Table 2).

Despite performing less work, iron-deficient HF subjects displayed more pronounced end-exercise drop of PCr and pH as compared with HF subjects without ID (Figure 2). Exercise-induced change in PCr correlated with more profound muscle acidification (r=0.74, P<0.0001) in ID HF, while in non-ID HF, there was no relation between the change of PCr and pH, similar to the healthy controls (r=0.03, P=0.94; Figure 3B).

In HF subjects, acidification of the muscle in proportion to change of PCr during exercise correlated with systemic iron stores estimated by circulating ferritin (Figure 3C).

Impact of Short-Term Iron Repletion on Muscle Bioenergetics

In 13 HF subjects with ID (age 60±15 years, 85% males, 69% non-CAD HF cause), examinations were repeated 1 month after the first examination after 2 doses of 500 mg intravenous iron therapy using FCM. HF patients with ID who did not receive intravenous iron did not differ (P>0.05) in clinical characteristics or iron status from those who received it (data not shown). As shown in Table 4, the FCM administration improved iron status, hemoglobin concentration, and maximal isometric force but produced no change in high-energy phosphates during rest, exercise, and recovery.

DISCUSSION

This study shows that the combination of HF and ID is associated with abnormalities in skeletal muscle metabolism and that ID may interact synergistically with HF to promote skeletal myopathy. Iron-deficient HF subjects were more symptomatic, had lower peak muscle strength, larger energetic depletion and more pronounced acidification of the muscle during exercise, consistent with metabolic shift towards anaero-

Table 3. Determinants of Muscle Bioenergetics in HF Group in the Recovery Phase (n=44)

	Q_{max}			V_{PCr}			τ_{PCr}		
	β (SE)	r^2	*P* Value	β (SE)	r^2	*P* Value	β (SE)	r^2	*P* Value
Age (per 10 y)	−0.07 (0.02)	0.32	0.0003	−0.03 (0.01)	0.21	0.0005	7.5 (4.6)	0.07	0.11
Sex (F)	0.04 (0.03)	0.03	0.27	0.01 (0.02)	0.01	0.60	−3.7 (8.7)	0.01	0.67
No diabetes mellitus	0.07 (0.04)	0.10	0.05	0.03 (0.02)	0.06	0.13	−12 (8.8)	0.05	0.19
No iron deficiency	−0.02 (0.04)	0.01	0.59	−0.03 (0.02)	0.05	0.18	−11 (9.2)	0.03	0.24
BMI (per 5 kg·m^{-2})	−0.08 (0.17)	0.15	0.02	−0.04 (0.01)	0.14	0.02	6.6 (7.7)	0.02	0.40
Cause of HF (CAD)	−0.05 (0.03)	0.05	0.18	−0.03 (0.02)	0.04	0.04	−4.1 (8.4)	0.01	0.62
Q_{max} (per 0.1 mmol·s^{-1})	…	…	…	0.05 (0.003)	0.88	<0.0001	−10.4 (3.4)	0.21	0.004

Univariate model. β indicates least squares linear regression coefficient; BMI, body mass index; CAD, coronary artery disease; Q_{max}, maximal oxidative flux; τ_{PCr}, time constant of PCr recovery; and V_{PCr}, initial rate of PCr recovery.

bic glycolysis. Because exercise muscle acidification is known to be related to early fatigue,[17,31] the results support mechanistic link between ID and exercise intolerance in HF. However, although 1 month of therapy with intravenous iron administration corrected iron levels, improved erythropoiesis response, and enhanced muscle strength, it did not change muscle bioenergetics. Collectively, these data reinforce the importance of skeletal myopathy in HF, particularly in the presence of ID, and they suggest that ID-induced changes in skeletal muscle bioenergetics may require more intensive, more sustained or more muscle-targeting iron therapy. Alternative metrics, beyond conventional measures of systemic iron homeostasis, might be needed to optimally reflect muscle tissue iron stores.

Skeletal Myopathy in HF

Patients with chronic HF often complain of diminished skeletal muscle performance. Multiple mechanisms, such as hypoperfusion, deconditioning, inflammation and increased oxidative stress lead to muscle atrophy and weakness, contributing to HF symptoms.[13,15] Here we provide an evidence for intrinsic, muscle mass independent, bioenergetic basis of HF-related myopathy. The time constant of PCr recovery (τ_{PCr}), an indicator ATP synthesis by oxidative phosphorylation, was more impaired in HF subjects than in controls, confirming previous studies.[15,32,33] The reasons for slower PCr recovery could be inherent mitochondrial dysfunction or inadequate delivery of substrates and oxygen to the mitochondria.[33–35] We observed a strong correlation between age and Q_{max}, which may be explained by mitochondrial senescence[36] or microvascular dysfunction.[37] These changes were restricted to the HF group, identifying unique adverse effects of HF and aging on skeletal muscle, and they suggest that greater therapeutic attention ought to be paid to changes in skeletal muscle in older adults with HF.[38,39]

Synergistic Action of ID and HF on Skeletal Muscle

In recent clinical trials, iron repletion improved quality of life in patients with HF and ID[24,40] spurring greater interest in the role of iron in the skeletal myopathy of HF. However, to date, there have been no human data linking HF-related disorders of iron homeostasis to skeletal muscle dysfunction.[18] In our study, iron-deficient HF subjects displayed more profound decline of energetic reserve (PCr) and more severe muscle acidification at low-level exercise when compared with nonsideropenic HF. These alterations may contribute to premature muscle fatigue and decreased exercise tolerance, even if net muscle oxidative phosphorylation (reflected by Q_{max}) is preserved.[41] Previously, Wilson et al[42] and Massie et al[43] used a similar approach and identified a subgroup of HF patients with more profound drop of pH for given workload. Although iron status was not assessed in these reports, it is possible that those HF patients were in fact iron deficient and ID accounted for their greater myopathy. In summary, presented data support the idea that ID and HF act in synergistically deleterious fashion on skeletal muscle, resulting in metabolic myopathy. Nevertheless, the causality still needs to be verified by further interventional studies.

ID and Muscle Metabolism

The biological explanations for the observed abnormalities are not established but may involve greater expression of lactate-generating IIb type fibers[16] or biochemical adjustments in muscle to the presence of ID and HF that lead to impaired mitochondrial function. In a rodent model of nutritional sideropenia, ID led to profound anemia, decreased skeletal muscle mitochondrial enzyme activities, and diminished exercise capacity.[6,9,44] Impaired exercise capacity of ID rats persisted even after transfusion, suggesting effects that were anemia independent.[9] Iron-deficient animals developed more rapid elevation of muscle lactate[44] and lower intracellular pH[45], which may be indicative of a switch towards

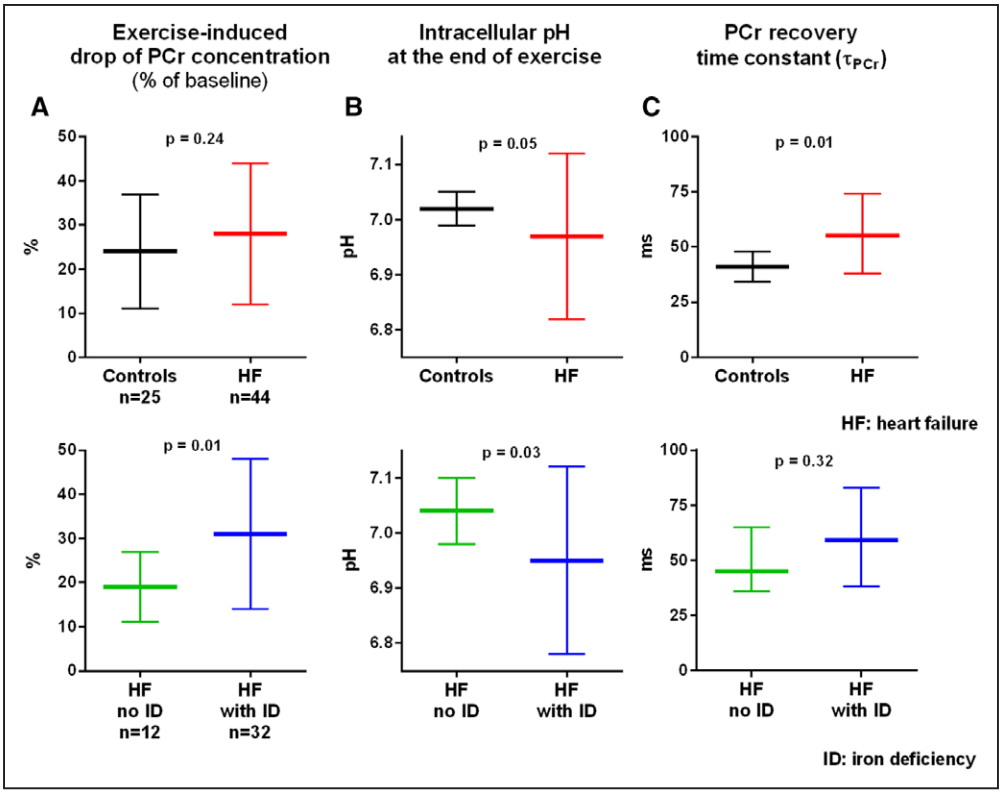

Figure 2. The impact of heart failure (HF) and iron deficiency (ID) on exercising calf muscle bioenergetics.
A, The effect on exercise-induced drop of phosphocreatine (PCr). **B**, The effect on calf muscle intracellular pH at the end of exercise. **C**, The effect on PCr recovery time constant (τ_{PCr}). Plots show group means±SD compared by unpaired *t* test (**A**, **B**) or median (interquartile range) compared by Wilcoxon test (**C**).

anaerobic glycolysis due to diminished oxidative phosphorylation in muscle tissue.[7,46] In these ID animal models, iron administration quickly restored hemoglobin levels, but recovery of muscle metabolism had slower kinetics and did not completely reverse.[6,45]

In humans, the iron deficit is usually less profound than in animal experiments,[45] and the relations between ID and muscle dysfunction are less consistent. In a muscle biopsy study performed in volunteers with endemic sideropenia, muscle myoglobin content, and mitochondrial enzyme concentrations were preserved.[12] In healthy volunteers who underwent repeated venesections to induce severe ID over the course of 9 weeks, muscle biopsies did not show diminished capacity of glycolytic, oxidative, and iron-dependent enzymes.[11] Females with normal cardiac function but severe ID because of chronic blood loss, displayed preserved mitochondrial ATP synthesis and normal postexercise PCr recovery time constant in skeletal muscle.[10] These human studies indicate that ID alone (though not as extreme as in experimental animals) may be insufficient to induce changes in muscle bioenergetics.

The relations between iron content and mitochondrial function were recently analyzed in cardiac muscle speci-

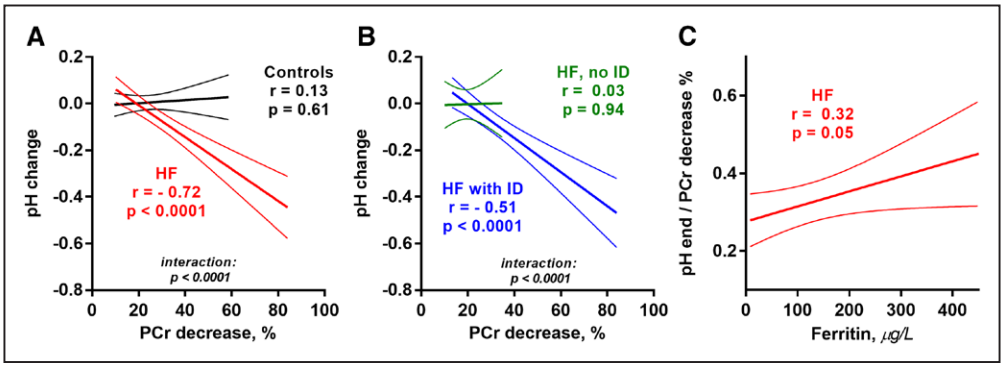

Figure 3. The relations between exercise-induced muscle energetic depletion (% drop of baseline phosphocreatine [PCr]), acidification (drop of pH), and iron status.
A, In patients with heart failure (HF) and in controls. **B**, In patients with HF with or without iron deficiency (ID). **C**, Relation between pH at the end of exercise normalized to exercise-induced energetic depletion and total iron stores, measured by ferritin. Lines are regression lines and 95% CIs, *r*=Pearson correlation coefficient.

Table 4. Effect of Intravenous Iron Administration in HF Patients (n=13)

	Before Intravenous Iron	After Intravenous Iron	Change	*P* Value
LV ejection fraction, %	28±11	29±13	0.5 (–2.4, 3.6)	0.72
BNP,* ng·L^{-1}	993 (638, 1945)	877 (627, 1683)	–9% (–28%, 18%)	0.77
Iron metabolism				
Hemoglobin, g·L^{-1}	125±13	133±15	8.1 (2.0, 14.3)	0.013
Mean cell volume, fL	87±5.5	90±3.7	3.0 (1.1, 4.8)	0.005
Ferritin,* µg·L^{-1}	68 (50, 117)	317 (207, 600)	403% (236%, 717%)	<0.001
Transferrin, g·L^{-1}	3.1±0.5	2.7±0.4	–0.5 (–0.8, –0.2)	0.001
Transferrin sat,* %	10 (7, 23)	18 (15, 31)	87% (42%, 155%)	<0.001
Serum iron,* µg·dL^{-1}	48 (40, 85)	75 (58, 112)	66% (30%, 102%)	0.001
Rest bioenergetics				
PCr	0.50±0.03	0.50±0.02	–0.01 (–0.02, 0.01)	0.55
Pi	0.08±0.01	0.08±0.01	–0.01 (–0.01, 0.01)	0.51
ATP	0.08±0.01	0.08±0.01	–0.01 (–0.01, 0.01)	0.64
ATP/Pi	1.03±0.24	1.00±0.27	–0.03 (–0.24, 0.18)	0.51
pH rest	7.03±0.02	7.03±0.03	0.01 (–0.02, 0.03)	0.78
Exercise and recovery bioenergetics				
Work, W	2.4±1.1	2.4±1.4	0.1 (–0.6, 0.8)	0.95
Peak isometric force,* N	349 (272, 460)	384 (293, 518)	5% (2%, 25%)	0.048
PCr drop, %	31±22	31±17	–1.8 (–8.7, 3.2)	0.94
τ_{PCr},* ms	63 (38, 125)	65 (49, 101)	0.1% (–0.1%, 0.3%)	0.49
V_{PCr},* mmol·s^{-1}	0.25 (0.19, 0.38)	0.24 (0.18, 0.38)	0.0% (–0.2%, 0.3%)	0.77
Q_{max}, mmol·s^{-1}	0.47 (0.36, 0.70)	0.46 (0.35, 0.67)	0.0% (–0.2%, 0.2%)	0.73
pH end	6.90±0.20	6.92±0.17	0.02 (–0.04, 0.10)	0.41
ADP end, µmol·L^{-1}	36±11	34±12	–0.8 (–8.1, 6.5)	0.98

PCr, Pi, and ATP are expressed in relative units (fraction of the total spectral intensity). *P* value of paired *t* test on log-transformed data. ADP indicates adenosine diphosphate; ATP, adenosine triphosphate; BNP, B-type natriuretic peptide; HF, heart failure; ID, iron deficiency; LV, left ventricular; PCr, phosphocreatine; Pi, inorganic phosphate; Q_{max}, maximal oxidative flux; τ_{PCr}, time constant of PCr recovery; and V_{PCr}, initial rate of PCr recovery.

*Values are geometric means (95%CI) with relative % change (95%CI); all other are arithmetic means (±SD) with absolute change (95%CI).

mens from HF patients or controls.[7] Patients with HF and myocardial ID had diminished enzymatic activities in the citric acid cycle, whereas enzyme activities of the respiratory chain were not affected. The results indicate that the rate of ATP formation from acetyl-coenzyme A processing by the citric acid cycle may be reduced by ID, whereas ATP formation from glycolysis may not. Increased glycolysis and lactate formation during increased demand

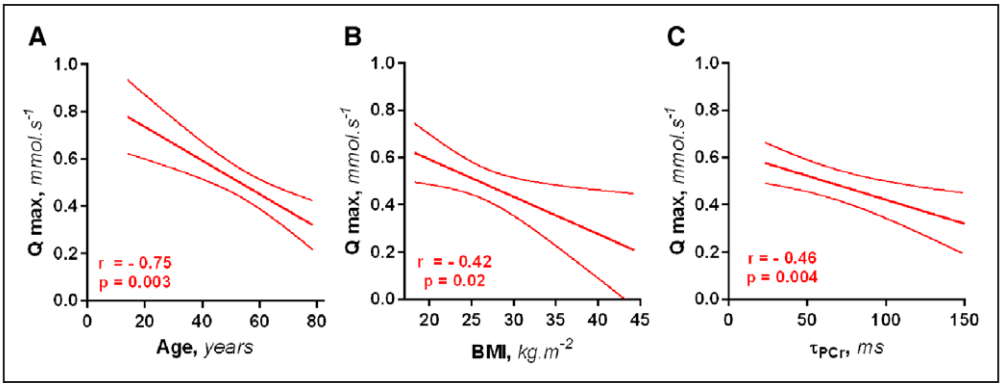

Figure 4. The determinants of maximal oxidative flux (Q_{max}) in heart failure subjects.
Q_{max} was significantly affected by age (**A**) and body mass index (BMI, **B**). Q_{max} significantly impacted PCr recovery time constant (τ_{PCr}), the time constant of phosphocreatine (PCr) recovery (**C**). Lines are regression lines and 95% CIs, *r*=Pearson correlation coefficient.

may compensate for slower ATP production via oxidative phosphorylation.[46] This mechanism may also operate in skeletal muscle and contribute to greater muscle acidosis in ID-HF patients, as observed in present study.

Effect of Short-Term Iron Therapy on Muscle Bioenergetics

The lack of change of muscle bioenergetics after iron supplementation with FCM in HF subjects with ID indicates that if an improvement of muscle bioenergetics occurs, it requires more time than the correction of anemia, and certainly >1 month. In animal experiments with radio-labeled iron FCM formulation, delivered iron is immediately taken up by macrophages in the bone marrow and in the reticuloendothelial system, where it is stored or incorporated into rapidly dividing hemopoietic cells.[47] The kinetics of iron incorporation into human muscles is unknown, but there may be considerable time lag needed for secondary internal redistribution of iron from liver stores into skeletal muscle, with greater time needed for metabolic reprogramming. Correspondingly, in the CONFIRM-HF trial (Ferric Carboxymaltose Evaluation on Performance in Patients With Iron Deficiency in Combination With Chronic Heart Failure), the first significant improvement in fatigue was not detectable until after 12 weeks of FCM therapy.[40] Perhaps other iron formulations may display different kinetics of iron incorporation into the skeletal muscle. Small observed improvements of symptoms and muscle strength after iron therapy in our study may be related to correction of anemia, similar to what is seen in darbepoetin-treated HF patients.[48]

Limitations

Workload was not adjusted to body size but fixed. Mandatory low-level load was used in all subjects in a previously validated protocol.[25] Differences in body size can confound the results; however, the groups were selected in such a way that both HF and control subjects had similar BMI. We measured only peak muscle isometric force, which is a crude parameter of muscle function. More detailed dynamic analysis of muscle performance and use of individually adjusted workloads may provide more accurate characterization of muscle bioenergetics. Due to a lack of preexisting human studies, and given the safety concerns of HF patients without defibrillators, we selected a short observation period of 1 month, assuming that muscle iron metabolism will respond to intravenous iron in a similar time frame as the bone marrow. It is possible that a longer observation period or other iron preparation might have shown significant improvements in the muscle metabolism in patients with HF and ID. The small sample size of the intervention study prevented analysis of relations of biochemical improvements to functional/clinical improvements. The effect of iron on muscle bioenergetics was not tested compared with placebo group, but our results do not indicate relevant change after 1 month.

Conclusions

In conclusion, ID may contribute to skeletal muscle abnormalities that are often observed in patients with chronic HF. Although maximal mitochondrial activity was not impaired, HF patients with ID demonstrated more profound drops in PCr and pH than non-ID HF patients, consistent with more pronounced anaerobic glycolysis. This acidification of the muscle at the peak of exercise may contribute to fatigue and impaired muscle performance. Maximal mitochondrial respiration declined markedly with aging in HF subjects, but not controls, emphasizing that HF patients may stand to benefit more from interventions targeted to skeletal muscle with aging. Improvements in skeletal muscle bioenergetic abnormalities in HF patients with ID may require longer durations of FCM treatment as compared with repletion of internal iron stores or red blood counts, and novel markers of tissue iron content may be needed to judge the adequacy of iron repletion as it affects skeletal muscle.

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Correspondence

Vojtech Melenovsky, MD, PhD, Department of Cardiology, Institute for Clinical and Experimental Medicine, IKEM, Videnska 1958/9, Prague 4, 140 28, Czech Republic. Email vojtech.melenovsky@ikem.cz

Affiliations

Department of Cardiology (V.M., K.H., J.K.) and Diagnostic and Interventional Radiology (P.S., M.D., M.H.), Institute for Clinical and Experimental Medicine, IKEM, Prague, Czech Republic. Department of Cardiovascular Disease, Mayo Clinic, Rochester, MN (B.A.B.). BIOCEV, First Faculty of Medicine, Charles University, Vestec, Czech Republic (J.P.).

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Disclosures

None.

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