BACKGROUND: Among central and peripheral factors contributing to exercise intolerance (EI) in heart failure (HF), the extent to which skeletal muscle (SM) energy metabolic abnormalities occur and contribute to EI and increased fatigability in HF patients with reduced or preserved ejection fraction (HFrEF and HFrEF, respectively) are not known. An energetic plantar flexion exercise fatigability test and magnetic resonance spectroscopy were used to probe the mechanistic in vivo relationships among SM high-energy phosphate concentrations, mitochondrial function, and EI in HFrEF and HFrEF patients and in healthy controls.

METHODS AND RESULTS: Resting SM high-energy phosphate concentrations and ATP flux rates were normal in HFrEF and HFrEF patients. Fatigue occurred at similar SM energetic levels in all subjects, consistent with a common SM energetic limit. Importantly, HFrEF New York Heart Association class II–III patients with EI and high fatigability exhibited significantly faster rates of exercise-induced high-energy phosphate decline than did HFrEF patients with low fatigability (New York Heart Association class I), despite similar left ventricular ejection fractions. HFrEF patients exhibited severe EI, the most rapid rates of high-energy phosphate depletion during exercise, and impaired maximal oxidative capacity.

CONCLUSIONS: Symptomatic fatigue during plantar flexion exercise occurs at a common energetic limit in all subjects. HFrEF and HFrEF patients with EI and increased fatigability manifest early, rapid exercise-induced declines in SM high-energy phosphates and reduced oxidative capacity compared with healthy and low-fatigability HF patients, suggesting that SM metabolism is a potentially important target for future HF treatment strategies.
WHAT IS KNOWN

- Skeletal muscle energetics were studied with $^{31}$P magnetic resonance spectroscopy during plantar flexion exercise in healthy subjects and heart failure (HF) patients with and without exercise intolerance (EI).
- Planar flexion exercise performance correlated with separate 6-minute walk and peak VO$_2$ testing. Skeletal muscle energetic levels in heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction patients were normal at rest.
- HFrEF New York Heart Association class II–III patients with EI and high fatigability exhibited significantly faster rates of exercise-induced high-energy phosphate decline than did HFpEF patients with low fatigability (New York Heart Association class I).
- Heart failure with preserved ejection fraction patients exhibited severe EI, the most rapid rates of high-energy phosphate depletion during exercise and impaired maximal oxidative capacity.

WHAT THE STUDY ADDS

- EI and exertional fatigue are hallmark symptoms of HF and peripheral factors may contribute to EI in HF.
- This study showed that symptomatic fatigue during plantar flexion exercise occurs at a common energetic limit in healthy and HF subjects.
- HFrEF and heart failure with preserved ejection fraction patients with EI and increased fatigability manifest early, rapid exercise-induced declines in skeletal muscle high-energy phosphates and reduced oxidative capacity compared with healthy and low-fatigability HF patients.
- These observations suggest that skeletal muscle metabolism may be a potentially important target for future HFpEF and heart failure with preserved ejection fraction treatment strategies.

Exercise intolerance (EI) and exertional fatigue are hallmark symptoms of heart failure (HF) and are associated with increased disability and mortality. HF is a multidimensional problem with both central (ie, cardiac) and peripheral (ie, skeletal muscle [SM] and neurological) factors contributing to EI. Observations that EI can persist for months after normalization of cardiac output after cardiac transplantation and that exercise training improves exercise tolerance in HF patients without improving exercise cardiac output both suggest that peripheral factors may be important contributors to EI and increased fatigability in HF.

Fatigue is often conceptualized as an energy-deficient state, and this is certainly the case in skeletal muscle, where ATP is absolutely required to fuel muscle contractile function. Skeletal muscle fatigue in isolated preparations is related to high-energy phosphate depletion, reduced energy release from ATP hydrolysis ($\Delta G_{ATP}$), and accumulation of inorganic phosphate (Pi) from ATP degradation. Although impaired ATP metabolism and energy deprivation play an important role in muscle fatigue and weakness in metabolic myopathies and muscular dystrophies, the role of skeletal muscle energy deprivation in HF is not well characterized. Phosphorous ($^{31}$P) magnetic resonance spectroscopy (MRS) studies enable repeated assessments of ATP and creatine phosphate (PCr) concentrations and turnover rates during exercise that are not possible with muscle biopsy specimens. However, to date, $^{31}$P MRS studies in patients with HF and a reduced ejection fraction (HFrEF) have produced conflicting results with some, reporting reduced SM high-energy phosphate ratios during submaximal exercise. In HF with preserved ejection fraction (HFpEF), reduced skeletal muscle type 1 fibers and mitochondrial content have been observed and a single previous $^{31}$P MRS skeletal muscle study included only 2 patients.

To evaluate the role that SM metabolism plays in HF EI, it is important to recognize that fatigue may occur at markedly different exercise durations or intensities. The term fatigability was introduced to relate the symptom of tiredness or fatigue to the level, duration, or intensity of the exercise that induced the symptom. Thus, a complete SM metabolic profile during exercise requires assessments at rest, the rate of change of those parameters during exercise, measures at a common duration or intensity, and again at final fatigue. Furthermore, SM parameters recorded during treadmill or bicycle exercise are likely influenced by central mediated hemodynamic shifts, making it difficult to determine whether any changes in the SM parameters are secondary to such global factors or to intrinsic, primary SM-associated metabolic abnormalities. Finally, important metabolic information should not only include the ratios of high-energy phosphate compounds as reported previously but also their absolute concentrations and rates of synthesis.

In the present study, we exploit an energetic fatigability test that combines graded plantar (small muscle) flexion exercise (PFE) performed in conjunction with repeated noninvasive $^{31}$P MRS measures of muscle high-energy phosphates, inorganic phosphate, and intracellular pH at rest and during progressive exercise stages performed to fatigue. Four subject cohorts were studied (1) those with HFrEF and EI as defined by New York Heart Association (NYHA) class II or III; (2) those with HFpEF without EI as defined by NYHA class 1, but with left ventricular ejection fractions (EF) matched to those in group 1; (3) patients with HFpEF; and (4) healthy subjects. The findings are the first to quantify SM metabolism in HFpEF patients and are consistent with the hypothesis that SM
metabolic abnormalities play an important mechanistic role in EI in HFrEF and HfPEF patients.

METHODS
The Johns Hopkins Institutional Review Board approved all human studies. All subjects gave informed written consent after explanation of the study and protocol.

Subjects
Eleven healthy subjects (6 women, age 51±7 years) with no history of hypertension, diabetes mellitus, or of heart or vascular disease served as controls. HF patients had a clinical diagnosis of chronic HF and included 20 subjects with HFrEF (EF ≤40%), 7 of whom had NYHA class I symptoms (2 women, age 43±14 years) and 13 with NYHA class II or III symptoms (7 women, age 52±11 years). Twelve other patients had chronic HfPEF (8 women, age 62±11), as defined by Framingham criteria with EF ≥50%23 (see Data Supplement for additional details).

Study Protocol
All subjects underwent conventional magnetic resonance imaging (for determination of muscle and fat content) and 31P MRS (for energetics) at rest, during graded multistage PFE (Figure I in the Data Supplement), and during the post exercise recovery period in a clinical 3 T magnetic resonance imaging system. Relative and absolute concentrations of high-energy phosphates were measured as were ATP kinetics with 31P magnetization transfer techniques. Fatigue symptoms were recorded at each stage using an 11-point Borg scale. Exercise was terminated when the subject was unable to exercise further. In addition, an upright bicycle ergometry cardiopulmonary exercise test to exhaustion to measure peak VO2 and a 6-minute walk (6MW) were performed (see Data Supplement for details of protocol and statistical analysis).

RESULTS
Patient Characteristics
HfPEF patients were older than NYHA class I HFrEF patients (Table). Symptomatic HF patients (HFrEF NYHA class II–III and HfPEF) were more obese than healthy subjects. As expected, symptomatic HF patients (HFrEF NYHA class II–III and HfPEF) had significant EI with reduced mean 6MW distances and peak VO2 as compared with healthy subjects (Table). However, NYHA class I HFrEF patients had nearly normal functional measures with 6MW and peak VO2 similar to those of healthy subjects despite their significantly lower EF.

PFE Performance and Established Functional Parameters
Mean PFE time, maximum exercise weight, and total work were reduced in HfPEF and HFrEF NYHA class II–III patients compared with healthy subjects and HfPEF patients with NYHA class I symptoms (Figure 1A). HfPEF patients exhibited the most EI during PFE with HfPEF and HFrEF NYHA class II–III patients able to perform only one third to one half of the total work performed by healthy subjects (Figure 1A). Of note, indices of PFE performance (exercise time, maximum exercise weight, and total work) correlated significantly with the more established, global functional indices of 6MW and peak VO2 (Figure 1B). Thus, PFE performance parallels accepted functional measures used in many HF studies. Similar correlations are observed when corrected for ideal body weight (Figure II in the Data Supplement), rather than true body weight, suggesting that the observed differences are not attributable to obesity.

31P MRS Energetic Fatigability Test
Representative images and spectra from a 31P MRS/magnetic resonance imaging energetic fatigability test are shown in Figure 2A through 2C. During exercise, there is depletion of PCr and accumulation of Pi, with intracellular acidification reflected by the change in chemical shift of the Pi resonance (Figure 2C). ATP synthesis through creatine kinase (CK) and that from Pi were calculated from 31P magnetization transfer spectra at rest (Figure 2D through 2G). The time course of energetic changes and fatigue symptoms are shown for a healthy subject with low fatigability (Figure 3A) and a HF patient with high fatigability (Figure 3B). Note that SM energetics can be quantified with a high temporal resolution (2s), PCr is progressively depleted during staged-exercise while ATP is preserved, and, critically, the rate of PCr decline is more rapid in the subject with high fatigability. Note that both subjects reach a similar subjective level of fatigue measured by the Borg scale but at different workloads and exercise durations.

Skeletal Muscle Energetics at Rest, Exercise, and Fatigue
During baseline resting conditions, there were no significant differences in ATP synthesis rates from PCr through CK and from Pi (Figure 2F and 2G) or other SM energetic parameters among the 4 groups (Figure 4A through 4F). Thus, resting SM high-energy phosphates and energetics are normal in HF patients. However, phosphodiesters, a byproduct of phospholipid catabolism, are increased. Despite differences in exercise time and work, energetic changes are similar at the point of fatigue in all groups (Figure 4G through 4K) with comparable PCr depletion, Pi, and ADP accumulation, intracellular acidosis, and change in ΔG_ATP. Thus, the SM energetic profile is similar in healthy subjects and HF patients before exercise and at the point of fatigue (Figure 4), although, again, the times to fatigue significantly differ (Figure 1).
Rate of High-Energy Phosphate Decline During Exercise and Postexercise Rate of Recovery

Although SM high-energy phosphates are comparable at rest and at the time of fatigue in the 4 groups, the rates of PCr decline and Pi accumulation differ significantly. Specifically, the normalized rates of PCr decline during PFE were greater in HFpEF ($P<0.001$) and HFrEF NYHA class II–III patients ($P<0.005$) than in healthy subjects (Figure 5A). Because the rate averaged over all of exercise could be impacted by reduced cardiac-derived SM perfusion in HF patients at peak exercise, we also measured high-energy phosphate decline during the earliest 4 minutes of low-level exercise (Figure 5B). The initial rates of high-energy phosphate decline were still significantly greater in HFrEF II–III ($P<0.05$) and HFpEF patients ($P<0.001$) than in healthy subjects. There is a strong correlation between the average rate of PCr decline during PFE and the maximum exercise time (Figure 5C; $R^2=0.83; P<0.001$), indicating that the accelerated exercise-induced SM high-energy phosphate loss in symptomatic HFrEF and HFpEF patients is closely related to their EI.

The rate of PCr recovery after exercise directly reflects oxidative rephosphorylation of creatine and is related to maximal mitochondrial oxidative capacity.24 The time for PCr recovery was significantly delayed (Figure 6A) and maximum oxidative capacity reduced (Figure 6B) in HFpEF and HFrEF II–III patients compared with healthy subjects, indicating impaired mitochondrial function in the former 2 HF groups. In addition, maximum oxidative capacity in HFrEF patients with EI (NYHA class II–III) was half that of the NYHA class I HFrEF patients despite comparable mean EFs. This indicates that impaired SM mitochondrial function is more closely related to EI and HF

### Table. Demographics

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<th>HFpEF (n=12)</th>
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<td>2231±709</td>
<td>1710±613*†</td>
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Data are means±SD. 6MW indicates 6-min walk; ACE, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; ASA, aspirin; BMI, body mass index; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; Peak VO$_2$, peak oxygen consumption on cardiopulmonary exercise test testing; and RER, respiratory exchange ratio.

* $P<0.05$ vs HFpEF.
† $P<0.05$ vs healthy.
‡ $P<0.001$ vs healthy.
§ $P<0.02$ vs healthy.
symptoms than is EF in these HFrEF patients. Thus, symptomatic HFrEF patients with EI and HFrEF patients exhibit an accelerated loss of SM high-energy phosphates commencing during early exercise, before we expect blood flow to be limiting during small muscle exercise. These patients also exhibited impaired mitochondrial oxidative capacity compared with healthy subjects and with HFrEF patients with nearly normal exercise tolerance.

**Skeletal Muscle Fat Content**

Because obesity is associated with the development of both HFrEF and HFrEF and because obesity-associated lipid accumulation and lipotoxicity are thought to adversely affect cardiac muscle function, we also measured fat content in SM. Muscle fat fraction is non-significantly increased in HFrEF patients compared with that of healthy subjects and did not differ between HFrEF NYHA class I and class II–III patients (Figure 7). In contrast, SM fat fraction is increased almost 3-fold in HFpEF patients compared with healthy subjects (Figure 7). However, no correlations between muscle fat content and muscle mass and high-energy phosphate decline during exercise or during post exercise recovery are observed (Figure IV in the Data Supplement).

**DISCUSSION**

**SM High-Energy Phosphate Metabolism and EI in HF**

We evaluated SM fatigability in HF patients with and without EI using an approach that allowed simultaneous assessment of fatigue symptoms, lower-extremity exercise capacity, and SM energetics at rest, matched workloads and fatigue. Exercise duration and work performed with this energetic fatigability stress test correlated with conventional functional measures...
of 6MW and exercise peak VO₂ (Figure 1). The novel findings are that all subjects fatigue at a common SM energetic level but that a faster rate of energetic decline distinguishes HF patients with EI from those with normal exercise tolerance. Of the cohorts studied, HFpEF patients exhibit the most dramatic SM energetic changes (Figures 3, 5, and 6).

Reduced skeletal muscle PCr/ATP and PCr/Pi ratios were previously observed with 31P MRS in HFrEF patients during submaximal steady-state exercise in most,12–14 but not all, prior studies.15–17 To our knowledge, the present study is the first to noninvasively quantify absolute concentrations of skeletal muscle ATP and PCr in these patient populations and to do so throughout a graded exercise regimen performed to fatigue. Importantly, because the high-energy phosphate ratio of PCr/ATP may not change with concomitant depletion in both PCr and ATP, the present absolute HEP results demonstrate that SM ATP and PCr are not reduced in HFrEF or HFpEF patients at rest. Because the CK reaction is the primary muscle energy reserve reaction and because ATP flux through myocardial CK is reduced in human HF and predicts future clinical HF events,26 we also tested whether reduced skeletal muscle CK ATP synthesis is present in HF and contributes to EI. However, skeletal muscle ATP synthesis through CK at rest is not reduced in HF patients (Figure 2). Thus, resting skeletal muscle high-energy phosphate stores and CK reserve are not reduced and cannot account for EI in HFrEF or in HFpEF patients.

Framework for Energetic Fatigability in HF

Although fatigue is typically defined as a subjective sense of tiredness, fatigability is an arguably more useful term because it relates the symptom of tiredness to the level, duration, and intensity of the activity that induced the symptom.5 Fatigability is an important concept in HF because it can distinguish individuals who experience identical levels of symptomatic fatigue after different amounts of activity (eg, walking 5 m versus running a 10k race).

Because originally described by Eldadah,5 a healthy subject with low fatigability (Figure 8A, blue line)
achieves a higher level, duration, and intensity of work than does a subject with high fatigability (Figure 8A, red line) who experiences the same level of fatigue symptoms (fatigue limit) at less activity. Here, we expand the fatigability construct to include an energetic dimension (Figure 8B). If there was no energetic basis for increased fatigability in HF, then fatigue would be independent of SM high-energy phosphate stores (eg, less depletion, Figure 8B red line 1 if skeletal muscle energetics are not limiting). However, if an energetic component to HF fatigue exists, then those patients with high fatigability (Figure 8B red lines 2 and 3) and those with low fatigability (Figure 8B blue line) would fatigue at a comparable energetic limit or metabolic index. The current findings of a common energetic limit in HF patients and healthy subjects (Figure 4) that is reached more rapidly in HF

Figure 3. Time course of energetic changes and fatigue symptoms for a healthy subject with low fatigability (A) and for a heart failure (HF) patient with high fatigability (B).

In both cases, PCr is progressively depleted and Pi accumulates during staged-exercise while ATP is preserved. The rates of creatine phosphate (PCr) decrease and inorganic phosphate (Pi) increase are more rapid in the subject with high fatigability. Both subjects reached a similar subjective level of fatigue (Borg scale) and PCr depletion but at different workloads and exercise durations.

Figure 4. Skeletal muscle (SM) energetic parameters (PCr, Pi, pH, ADP, ΔG_ATP) and phosphodiester (PDE) during baseline resting conditions (A–F, left column) and at the point of fatigue (G–K, right column). There are no significant differences in the SM energetic parameters among the 4 groups at rest or at the point of fatigue (although PDE differed at baseline). Comparisons vs healthy subjects: §P<0.005, §§§P<0.001.
patients with increased fatigability because of a more rapid rate of SM energetic depletion (Figure 5 and red line 2 in Figure 8B) support the hypothesis that high-energy phosphate energetics is an important contributor to HF fatigability. In theory, a significant increase in [ADP], [Pi], or change in ΔG~ATP could impair myofilament function before ATP is fully depleted, as observed here. Note that the present observation of a common energetic limit involves exercise of a relatively small muscle group not likely limited by central hemodynamics in subjects studied and suggest the possibility that new interventions to delay or slow the SM energetic decline during exercise could reduce EI in both HFrEF and HFrEF.

Furthermore, the rates of high-energy phosphate decline during exercise are significantly faster in HFrEF patients with EI and high fatigability (NYHA class II–III) than are the rates in HFrEF patients with low fatigability (NYHA class I), despite similarly low EFs (Figure 5). HFrEF patients with severe EI exhibit the most rapid rates of high-energy phosphate depletion during exercise. Their maximal oxidative capacity, reflected in delayed high-energy phosphate recovery, is comparable to that of HFrEF NYHA class II–III patients (Figure 6). Indeed, the rate of high-energy phosphate decline during PFE is inversely related to exercise duration (Figure 5C). All of these observations are consistent with normal baseline energetics but a faster exercise-induced decline to a common energetic limit in symptomatic HF (Figure 8B, red line 2).

**Potential Mechanistic Factors Underlying Impaired SM Energetics in HF**

The more rapid decline of high-energy phosphate during exercise in HF patients with EI could be because of reduced ATP production and/or increased ATP utilization during exercise.

Reduced SM mitochondrial number and enzyme activity were previously reported in HFrEF and HFpEF. We observed reduced maximal oxidative capacity in HFrEF and HFpEF patients (Figure 6), consistent with most but not all pre-

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**Figure 5.** A, Normalized rate of creatine phosphate (PCr) decline during plantar flexion exercise (PFE). B, Initial normalized rate of PCr decline during the first 4 min of PFE, Correlation of maximal PFE t. Cime and rate of PCr decline. Significant differences are indicated: *P<0.05, §P<0.005, §§§P<0.001.

**Figure 6.** A, The rate of creatine phosphate (PCr) recovery after plantar flexion exercise (PFE) is significantly delayed in heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF) II–III patients compared with HFrEF class I and healthy subjects. B, Indices of maximal oxidative capacity (VmaxO2) are lower in New York Heart Association (NYHA) class II–II HFrEF and HFpEF patients than in the other 2 groups without exercise intolerance. Comparisons vs healthy subjects: §P<0.005, §§P<0.002. Comparisons vs HFrEF NYHA class I patients: ***P<0.01.
vious reports in HFrEF patients. We think these to be the first findings of significantly reduced maximal oxidative capacity in HFpEF. Although increased ATP consumption during exercise could occur at a matched workload in HF because of reduced muscle mass or inefficiency, we did not observe significant reductions in muscle mass in these patients (Figure III in the Data Supplement).

Reduced SM blood flow in HF patients could contribute to the more rapid energetic decline, catabolite accumulation, and mitochondrial dysfunction during exercise in HF patients. Although early studies showed reduced blood flow in HFrEF,30–32 subsequent studies during upper or lower-extremity exercise in HFrEF patients did not demonstrate reductions in extremity blood flow, tissue oxygenation, or myoglobin desaturation during exercise sufficient to cause SM energetic abnormalities.12,13,33,34 Such observations reduce the likelihood of deficiencies in oxygen delivery as an underlying cause and suggest instead a SM mitochondrial metabolic abnormality in HF. This is consistent with the present observation of a rapid PCr decline during the earliest minutes of small muscle group exercise when the workload is low (2 to 4 lbs; Figures 3 and 5B), and blood flow may not be limiting. Intrinsic SM energetic abnormalities that are closely related to EI in HF patients are consistent with the hypothesis that the myopathy of HF may not be driven so much by hemodynamic abnormalities but rather by changes in SM mitochondrial function, perhaps related to the milieu of neurohormonal factors, metabolites, and cytokines that bathe the skeletal muscle.35 Alternatively, if HF patients become sedentary because of reduced cardiac reserve,36 the inactivity, per se, may contribute to EI and SM mitochondrial abnormalities.

Although these are the first 31P MRS findings showing statistically significant skeletal muscle energetic abnormalities during exercise in HFpEF patients, Bhella et al22 described delayed PCr recovery in 2 HFpEF patients, a sample size that precluded quantitative comparisons. Although cardiac dysfunction is a significant contributor to EI in HFpEF patients,37,38 there is also evidence that peripheral factors contribute.22,39,40 Recently,
reduced peripheral oxygen extraction was identified as a major determinant of upright bicycle exercise capacity in HFrEF patients. This was attributed to limited diffusive oxygen transport, possibly because of greater diffusion distances or heterogeneity in the matching of regional flow to metabolic demand. However, the deficit in SM maximal oxidative capacity observed here in HFrEF patients could also contribute to reduced peripheral oxygen extraction during exercise. A meta-analysis of 6 randomized trials indicated that exercise training in HFrEF patients improves cardiorespiratory fitness, but this occurs without measurable changes in systolic or diastolic function. In 1 randomized trial, exercise training improved peak VO2 in HFrEF patients with only a minimal effect on cardiac output but with a significant increase in peak arterial-venous oxygen difference. Those findings indicate that peripheral factors contribute to improved exercise capacity with exercise training in HFrEF and by inference that peripheral mechanisms contribute to EI in HFrEF. All of these studies are consistent with the concept that HFrEF is a systemic syndrome affecting multiple organs, including SM.

The in vivo SM energetic abnormalities in HFrEF patients (ie, accelerated rate of PCr decline and of Pi and H+ accumulation during exercise in Figure 5 and reduced in vivo maximal oxidative capacity in Figure 6) are similar or more marked in the HFrEF, than in the HFrEF, group. Remarkably, the intermuscular (not subcutaneous) fat was increased an average 3- to 4-fold over that in healthy subjects (Figure 7), possibly reflecting common HFrEF comorbidities of obesity, diabetes mellitus, and dyslipidemia. An earlier study reported that increased intermuscular fat in HFrEF patients correlated with reduced peak VO2.

Limitations
The cohort size was small and derived from patients referred to a tertiary care center and so may not represent the more general population. Nonetheless, highly significant differences in SM energetics between those HF patients with, and without, EI were detected. The 31P MRS PFE fatigability examination is not intended to replace standard 6MW or peak VO2 testing but instead to provide insight into the role of SM energetic fatigue in HF patients. The correlation of these planar flexion findings with conventional measures of total body exercise capacity is of interest because it cannot be assumed that capacity of a small muscle mass and total body exercise are necessarily limited by the same processes. Lower-extremity blood flow, endothelial function, and muscle perfusion were not measured with magnetic resonance imaging during PFE because of motion artifacts, and systemic lactate measures were not obtained. Thus, we cannot exclude the possibility that reduced perfusion during exercise contributed to the observed metabolic declines during exercise in HF subjects with EI. It seems unlikely, though, that reduced perfusion alone is responsible for energetic decline during the first minutes of low-level exercise or reduced maximal oxidative capacity (VmaxPCr) during recovery. HFrEF patients in many studies including this one tend to be older, be more obese, and have greater NYHA symptoms and EI. These data do not demonstrate whether the more dramatic energetic changes in HFrEF patients are because of HFrEF per se or to these other factors. It will be important in future studies to study older, obese subjects without HF, asymptomatic HFrEF patients and to match NYHA symptoms and exercise tolerance in HFrEF and HFrEF populations.

Conclusions
To evaluate whether SM energetic abnormalities exist and contribute to EI in HF patients, we exploited a SM energetic fatigability test that is noninvasive, correlates with established functional measures (6MW and peak VO2), and allows studies of SM energetics at matched workloads and at fatigue. HFrEF patients with increased fatigability and EI exhibit more rapid exercise-induced declines in SM high-energy phosphates than do HFrEF patients with no EI, despite matched left ventricular ejection fractions. HFrEF patients in this study exhibit the most profound EI, energetic abnormalities, and rapid PCr depletion during exercise as well as increased muscle lipids. On average, all subjects fatigue at the same mean SM energetic state, suggesting a common energetic limit. Because of the relatively high plasticity and remodeling potential of skeletal muscle to respond to multiple stimuli, compared with cardiac muscle, these studies offer a new approach to quantify SM energetics in HF and suggest that interventions that augment SM metabolism may offer another treatment target for the EI and disability that markedly impair quality of life for both HFrEF and HFrEF patients.

SOURCES OF FUNDING
This study was supported by the following National Institutes of Health grants: HL61912, HL63030, and AG045634.

DISCLOSURES
After the study was completed, Dr K. Weiss became an employee of Philips Healthcare at which time he performed data analysis and article preparation. No financial support from Philips Healthcare was received for the study.

AFFILIATIONS
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FOOTNOTES

Received April 11, 2017; accepted June 16, 2017.


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Cir Heart Fail. 2017;10:e004129. DOI: 10.1161/CIRCHEARTFAILURE.117.004129

July 2017

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Circ Heart Fail. 2017;10:e004129. DOI: 10.1161/CIRCHEARTFAILURE.117.004129

July 2017

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Circ Heart Fail. 2017;10:
doi: 10.1161/CIRCHEARTFAILURE.117.004129
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/10/7/e004129

Data Supplement (unedited) at:
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SUPPLEMENTAL MATERIAL

Fatigability, exercise intolerance and abnormal skeletal muscle energetics in heart failure

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Running title: Heart failure fatigability and skeletal muscle energetics
Submission:
Word count: 2404

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SUPPLEMENTAL METHODS:

Study Design

The Johns Hopkins Institutional Review Board (IRB) approved all human studies. All subjects gave informed consent after explanation of the study and protocol. This was a prospective study in which $^{31}$P MRS and MRI data were collected and analyzed by investigators (KW, AE-S, YZ, MS) blinded to clinical status (NYHA class, peak VO$_2$, 6MW), and clinical indices were measured or adjudicated by clinicians (GP, SR, RGW) blinded to $^{31}$P MRS and MRI results.

Subjects

Eleven healthy subjects (6 women, age 51±7 years) had no history of hypertension, diabetes mellitus, or of heart or vascular disease. Heart failure patients had a clinical diagnosis of chronic HF and included 20 subjects with HFrEF (EF < 40%) 7 of whom had NYHA class I symptoms (2 women, age 43±14 years) and 13 with NYHA II or III symptoms (7 women, age 52±11 years). Twelve patients had chronic HFpEF (8 women, age 62±11), as defined by Framingham criteria with EF > 50%. None of the HF patients had coronary disease as defined by >50% luminal stenosis assessed by cardiac catheterization, computed tomography angiography (CTA) or by a positive stress nuclear or echocardiogram test. Other exclusion criteria included significant valvular or pericardial disease, infiltrative or hypertrophic cardiomyopathy, cor pulmonale, significant pulmonary disease, and peripheral vascular disease. Those patients who had a recent HF decompensation were only enrolled after at least two weeks of clinical stabilization. Patients with any contraindication to magnetic resonance (MR) scanning (i.e. pacemaker, implantable cardioverter defibrillator), an inability to lie flat or complete the MR study, pregnancy, or those receiving investigational drugs were excluded from this study.

Energetic Fatigability Stress Testing

All subjects underwent $^1$H MRI and $^{31}$P MRS during rest, and dynamic $^{31}$P MRS during both graded plantar flexion exercise (PFE) and post exercise recovery in a clinical 3T MRI system (Philips Healthcare, The Netherlands). Subjects were positioned half seated, feet first in the MRI system with the calf muscle (gastrocnemius, soleus) of one leg centered on a custom built transmit and receive $^{31}$P MRS coil (Supplemental Figure 1). All $^1$H MRI examinations were performed with the body coil in transmit and receive mode. To minimize exercise contributions from muscle other than the calf muscle the exercising leg was fixed to the table using soft straps.

After positioning of the subject, scout images were acquired to verify the positioning of the leg and the $^{31}$P MRS coil. During rest before the graded exercise test, muscle composition of the lower leg was estimated with a set of spin-spin relaxation ($T_2$)-weighted images in all subjects. In a subset of 18 subjects multi-slice water and fat selective imaging of the lower leg was performed using a multipoint Dixon sequence and the fat fraction results correlated with those obtained by $T_2$-weighted imaging.

PFE was performed using an MR compatible custom built device that consisted of a foot pedal mechanism to lift an adjustable weight (Fig S1). The weight was lifted with a frequency of 1Hz and the weight was increased at the beginning of each 120s stage, starting with 0.9kg in the first stage, adding 0.9kg for the second stage and 1.8kg for every further stage. Exercise was terminated when the subject reached the point of fatigue and was not able to exercise further. Dynamic $^{31}$P MRS acquisitions with a temporal resolution of 2s were started 120s before the beginning of exercise and were continued during and after exercise. Fatigue symptoms were recorded at each work stage with an 11 point Borg scale (0-10). During exercise the subject’s heart rate was monitored using a fingertip pulse oximetry or ECG device.
and recorded at every exercise stage. Blood pressure was measured and recorded at every exercise stage using a blood pressure cuff on the upper arm.

**1H MRI image acquisition and analysis**

All 1H imaging was performed using the body coil for signal transmission and reception. T2-weighted images (FOV = 220 x 220 x 175 mm³, resolution = 0.78 x 0.78 x 10 mm³, 16 slices, pulse repetition time (TR) = 2141 ms, echo time (TE) = 100 ms) were acquired before and after PFE. Multipoint Dixon images (FOV = 220 x 220 x 175 mm³, resolution = 0.78 x 0.78 x 10 mm³, 16 slices, TR = 500 ms, TE = 1.85, 5.3, 8.75, 12.2, 15.65 ms, flip angle = 20°) were acquired before PFE.

Images were processed in Matlab (version 2012b; Mathworks, Natick, MA). To estimate the muscular fat content a central slice from the Dixon fat images (when available) and T2-weighted images were selected. Calf muscles were segmented manually to remove bones and the subcutaneous fat layer. For Dixon images muscular fat content was estimated as described by Triplett et al.4. To allow comparison among groups the reported fat fraction was additionally calculated from the T2-weighted images that were available for all subjects. T2-weighted images were normalized by the signal intensity of the subcutaneous fat layer, and pixel values above a threshold of 10% in the calf muscle segment were averaged for fat fraction estimation.

**31P MRS data analysis**

Absolute concentrations of HEPs were estimated as previously described by El-Sharkawy et al.3. The unidirectional rate of ATP synthesis through the creatine kinase reaction (PCr to ATP) were analysed as previously described Schar et al.5. The unidirectional rate of ATP synthesis from Pi (Pi to ATP) was analysed the same way by estimating the magnetization of Pi instead of PCr in the γ-ATP saturated spectra and acquiring an additional control saturation scan referenced to the Pi resonance.

Dynamic 31P MRS data were fitted using the AMARES function of the jMRUI package6. To estimate HEP dynamics during exercise the last ten spectra obtained at baseline and at every exercise stage were averaged prior to fitting. Intracellular pH was calculated from the chemical shift difference of the PCr and Pi resonances7. Cytosolic ADP concentration was calculated according to

\[
[ADP] = \frac{[ATP][Cr]}{[PCr][H^+]K_{eq}}
\]

assuming that 15% of the total creatine is unphosphorylated at rest6, and an equilibrium constant of \(K_{eq} = 1.66 \times 10^{-9}\). Normalized rates of PCr decline during exercise were estimated by linear regression of the PCr concentration versus the individual work performed at every stage in every subject. Gibbs free energy of ATP hydrolysis (\(\Delta G_{-ATP}\)) was determined via10:

\[
\Delta G_{-ATP} = \Delta G_0 + R \cdot T \cdot \log \left( \frac{[ADP][Pi]}{[ATP]} \right)
\]

where \(\Delta G_0\) is the standard free energy change, R is the universal gas constant and T is the absolute temperature.

Post exercise recovery of PCr after the time point of fatigue was fitted using a mono-exponential function to estimate the individual recovery time of PCr. The calculation of the maximal oxidative capacity (\(V_{max_{PCR}}\)) was based on Michaelis-Menten kinetics with a \(Km\) of 25µM11:

\[
V_{max_{PCR}} = \frac{V_{i_{PCR}}}{1 + \frac{Km}{[ADP]_{end}}}
\]

where [ADP]_{end} is the ADP concentration at end of exercise and \(V_{i_{PCR}}\) is the initial rate of PCr recovery according to:
\[ V_{i_{PCr}} = \frac{1}{\tau_{PCr}} \Delta PCr \]

With \( \tau_{PCr} \) being the estimated recovery time of \( PCr \) and \( \Delta PCr \) the concentration difference of \( PCr \) between end exercise and baseline conditions during rest.

Finally, some small amount of \( ^{31}P \) signal may derive from non-exercising muscle in small subjects or in those with atrophy. However, in those cases, the energetic abnormalities observed here would underestimate, rather than overestimate, the extent of exercise-induced energetic changes in HF. In addition, it is important to emphasize that the \( PCr \) recovery kinetics are independent of muscle mass and of the fraction of the muscle sampled.

**Cardiopulmonary exercise testing**

**Six-minute walk and Cardiopulmonary Exercise Testing (CPET):** Six-minute walk tests were performed on a 60-foot section of an enclosed corridor that is free of traffic and with chairs at each end to serve as markers. Patients sat quietly for 10 minutes before initiating the walk. The subjects were observed as they walked their maximum level distance during six minutes. At the conclusion, the distance and BORG symptoms were recorded\(^{12}\). CPET was performed at least 2-3 hours after taking medications with gas-exchange analysis on a MedGraphics Ultima cart during a cycle ergometer protocol with a 25-watt step increase in intensity every 3 minutes. Heart rate, blood pressure, symptoms, and the ECG were recorded at each stage and during recovery and BORG symptoms recorded during each exercise stage\(^{12-15}\). All patients exercise to exhaustion to a goal RER > 1.1 and an RPE > 18. Breath by breath oxygen consumption was measured and reported in 15 second averaged intervals. Peak oxygen consumption was defined as the average of the two highest oxygen uptake values during the last minute of exercise. Six-minute walk test was performed in all subjects except for one HFrEF patient and peak VO\(_2\) by CPET was measured in all subjects except in one HFrEF and three HFpEF patients.

Both peak oxygen consumption per kg of true body weight and per kg of ideal body weight were calculated and both showed similar correlations to total exercise work and total exercise time (Supplemental Figure 2).

**Statistical Methods:**

Continuous and categorical variables are presented as mean±standard deviation (SD) and numbers (percentages), respectively. We used Shapiro-Wilk test to assess the normality of all continuous variables. Normally distributed variables according to the Shapiro-Wilk test results were age, BMI, 6MW, baseline ADP, pH, \( \Delta G^{\sim}_{ATP} \) and CK and Pi->ATP flux, fatigue PCr, pH and \( \Delta G^{\sim}_{ATP} \). The normality test indicated that all other contiguous variables were not normally distributed. These variables were peak VO\(_2\); LVEF; baseline PCr, Pi, ATP and PDE; fatigue ATP and ADP; PCr recovery; maximum aerobic capacity; average and initial PCr decline rates; muscle fat fraction; total exercise time; and total work.

We used one-way analysis of variance (ANOVA) to determine differences among the four groups and the Student’s t-test for comparisons between HFrEF I group and HFrEF II-III group, when the data were normally distributed. Kruskal Wallis test and Wilcoxon Ranksum test were used for non-normally distributed data for comparisons among the four groups and the pair-wise testing, respectively. We used Bonferroni adjustment for all pairwise comparisons. A Spearman correlation test was used to calculate correlation coefficients. All Statistical analyses were performed using Stata Version 14 (StataCorp LP, College Station, Texas, USA). All tests of hypothesis were conducted at a confidence level of 0.95 under the two-sided alternative.
SUPPLEMENTAL REFERENCES


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Supplemental Figures:

Supplemental Figure 1: MRI-Plantar flexion exercise (PFE) setup wherein subjects are positioned prone (A) with the calf over the $^{31}$P MRS excitation/detection coil and moved to the center of the MRI scanner. (B) The foot is adjacent to a hinged foot-plate that allows plantar flexion and is attached to cables that run through the scanner to suspended adjustable weights outside the magnet bore. Adjustable wooden stops on the foot pedal (not shown) limit the extent of plantar flexion, which is monitored for consistency throughout. Velcro straps at the ankle, and below and above the knee prevent movement of the leg.
Supplemental Figure 2: To determine whether obesity and weight differences among the four groups contributed significantly to performance and the relationships between PFE performance measured as PFE Work (upper panels) and PFE Exercise Time (lower panels), CPET peak VO₂ corrected for actual body weight (left panels) was also calculated corrected for ideal body weight (right panels). The correlations between PFE performance parameters and CPET peak VO₂ were comparable when calculated using either actual body weight or ideal body weight, suggesting the findings are not primarily dependent on obesity in HF groups. The correlation coefficient Spearman’s rho is indicated by $r_s$. 

\[
\begin{align*}
\text{Healthy} & : y = 0.0581x + 0.1565, \quad R^2 = 0.3772 \\
\text{HFpEF} & : y = 0.0581x - 0.1829, \quad R^2 = 0.3287 \\
\text{HFpEF II-III} & : y = 11.808x + 339.04, \quad R^2 = 0.2972 \\
\text{HFrEF I} & : y = 11.775x + 271.14, \quad R^2 = 0.2572
\end{align*}
\]
Supplemental Figure 3: Muscle area measured from mid-slice MRI of lower leg (subcutaneous fat and bone excluded from cross-sectional area contours) (A) and in which the area was also corrected for muscle fat content (B). There was no significant difference in calf muscle mass among the four groups.
**Supplemental Figure 4:** Relationship between average rate of PCr decline during PFE and muscle fat fraction (left, $r^2=0.21$) and between post-exercise PCr recovery and muscle fat fraction (right, $r^2=0.05$).