Skeletal Muscle Alterations Are Exacerbated in Heart Failure With Reduced Compared With Preserved Ejection Fraction Mediated by Circulating Cytokines?

Martin Seiler, MSc*; T. Scott Bowen, PhD*; Natale Rolim, PhD; Maja-Theresa Dieterlen, PhD; Sarah Werner, BSc; Tomoya Hoshi, MD; Tina Fischer, BSc; Norman Mangner, MD; Axel Linke, MD; Gerhard Schuler, MD; Martin Halle, MD; Ulrik Wisloff, PhD; Volker Adams, PhD

Background—A greater understanding of the different underlying mechanisms between patients with heart failure with reduced (HFrEF) and with preserved (HFpEF) ejection fraction is urgently needed to better direct future treatment. However, although skeletal muscle impairments, potentially mediated by inflammatory cytokines, are common in both HFrEF and HFpEF, the underlying cellular and molecular alterations that exist between groups are yet to be systematically evaluated. The present study, therefore, used established animal models to compare whether alterations in skeletal muscle (limb and respiratory) were different between HFrEF and HFpEF, while further characterizing inflammatory cytokines.

Methods and Results—Rats were assigned to (1) HFrEF (ligation of the left coronary artery; n=8); (2) HFpEF (high-salt diet; n=10); (3) control (con: no intervention; n=7). Heart failure was confirmed by echocardiography and invasive measures. Soleus tissue in HFrEF, but not in HFpEF, showed a significant increase in markers of (1) muscle atrophy (ie, MuRF1, calpain, and ubiquitin proteasome); (2) oxidative stress (ie, higher nicotinamide adenine dinucleotide phosphate oxidase but lower antioxidative enzyme activities); (3) mitochondrial impairments (ie, a lower succinate dehydrogenase/lactate dehydrogenase ratio and peroxisome proliferator-activated receptor-γ coactivator-1α expression). The diaphragm remained largely unaffected between groups. Plasma concentrations of circulating cytokines were significantly increased in HFrEF for tumor necrosis factor-α, whereas interleukin-1β and interleukin-12 were higher in HFpEF.

Conclusions—Our findings suggest, for the first time, that skeletal muscle alterations are exacerbated in HFrEF compared with HFpEF, which predominantly reside in limb, rather than in respiratory, muscle. This disparity may be mediated, in part, by the different circulating inflammatory cytokines that were elevated between HFpEF and HFrEF. (Circ Heart Fail. 2016;9:e003027. DOI: 10.1161/CIRCHEARTFAILURE.116.003027.)

Key Words: diaphragm ■ heart failure ■ heart failure, diastolic ■ muscles ■ oxidative stress ■ proteasome endopeptidase complex

Heart failure (HF) is a major and growing public health concern, with estimates suggesting that ≈3.6 million patients are diagnosed every year in Europe alone. Of these, ≈60% of patients with HF have a reduced ejection fraction (HFrEF), whereas the remainder have a preserved ejection fraction (HFpEF). Importantly, patients with HFpEF fail to respond favorably to many pharmacological interventions that have otherwise proved beneficial to patients with HFrEF. This has widespread consequences for the treatment of patients with HF, suggesting 2 apparent cohorts exist that demonstrate a contrasting response to therapeutic interventions. As such, a greater understanding of the different underlying mechanisms acting between HFrEF and HFpEF may help better direct future treatment in this disease.

See Clinical Perspective

The main symptom observed in patients with HF is exercise intolerance, consequent not only to dyspnea but also to severe skeletal muscle weakness (both in the limb and respiratory systems), with the latter being a robust predictor of quality of life and prognosis. Interestingly, initial evidence from independent studies indicates that some but not all skeletal muscle.
Skeletal Muscle Alterations in HFrEF and HFpEF

Muscle alterations are similar between HFrEF and HFpEF when compared with controls. For example, HFrEF is well established to induce both a fiber atrophy and isoform shift, reduce contractile protein content, and lower mitochondrial capacity. These impairments are mediated by the combination of reduced anabolic (e.g., IGF-1) and elevated catabolic factors (e.g., atrogens and proteolytic activity). An increased reactive oxygen species (ROS) load, and greater inflammation driven by elevated concentrations of circulating cytokines (e.g., tumor necrosis factor [TNF]-α and interleukin [IL]-1β). In contrast, only a few studies have investigated skeletal muscle alterations in HFpEF with initial findings indicating fewer (or even opposite) changes may occur in comparison to HFrEF such as a reduced ROS load and lower proteolytic activity. Therefore, although preliminary evidence supports the suggestion that divergent skeletal muscle alterations may occur between HFrEF and HFpEF and thus contribute to disease progression, as of yet no direct comparison has been performed to answer this ambiguity.

The present study, therefore, used established animal models to directly compare whether molecular and cellular alterations in skeletal muscles (both limb and respiratory) were different between HFrEF and HFpEF. In addition, we also investigated whether circulating inflammatory cytokines were differentially expressed between the 2 conditions, as these may provide a potential mechanism to explain any such contrasting muscle alterations. A priori, we hypothesized that markers of atrophy and oxidative stress are more activated in the limb muscle of HFrEF when compared with that of HFpEF, whereas in the diaphragm, the differences between HFrEF and HFpEF are less pronounced.

Methods

Animals and Experimental Procedures
Female Sprague–Dawley rats (8 weeks old; n=8) underwent ligation of the left anterior descending coronary artery to induce a myocardial infarction and were euthanized 10 weeks later after development of HFrEF (confirmed by echocardiography and histology), as previously described. Female Dahl salt-sensitive rats (7 weeks old; n=10) were fed with a high-salt diet (8% NaCl) >28 weeks to induce HFpEF (confirmed by echocardiography and invasive pressure measurements). As recently described, the activity of chymotrypsin-like, trypsin-like, and peptidylglutamyl-peptide hydrolasing proteasome activity were assayed to provide an index of overall proteasome activity. Calpain activity was also assayed.

Plasma Analyses
A 12-plex rat cytokine assay was used to quantify in duplicates different cytokine concentrations from plasma samples using a Luminex 200 analyser (Merck Millipore, Darmstadt, Germany), in accordance with the manufacturer’s protocol (Bio-Rad, Munich, Germany).

Statistical Analyses
Data are presented as means±SEM. Because of small sample size, between-group differences were determined by nonparametric ANOVA (Kruskal–Wallis test), with post hoc test (Dunn multiple comparisons test). The pairwise P being reported are 2 sided. Significance was accepted as P<0.05. Analyses were performed by SPSS version 22 (SPSS Inc., Chicago, IL).

Results

Animal Characteristics
In HFrEF animals (n=8) that underwent ligation, echocardiography revealed significant systolic dysfunction showing an LVEF of 39±3%, whereas histological staining demonstrated an LV infarct size of 51±4%. In addition, fractional shortening was significantly reduced, whereas markers for ventricular dilation (left ventricular end-diastolic diameter and left ventricular end-systolic diameter) were increased (Figure 1). In contrast, HFpEF animals (n=10) demonstrated maintained LVEF of 70±4% but with diastolic dysfunction (82% increase in E/E and 116% increase in left ventricular end-diastolic diameter pressure) in combination with cardiac hypertrophy, as demonstrated by increased LV wall thickness and heart weight (Figure 1). As expected, however, Con rats (n=7) had no significant impairments to systolic or diastolic function.

Anabolic and Catabolic Markers

Soleus
Compared with Con, mRNA expression of the anabolic factor IGF-1 was significantly reduced by 77% in HFpEF
In HFrEF, IGF-1 was reduced by 66% but this did not reach statistical significance (Figure 2A). The catabolic protein MuRF1 (a muscle-specific ubiquitin E3 ligase) was significantly elevated in HFrEF by 46% but reduced in HfpEF by 41%, as compared with Con (Figure 2B). However, the protein expression of microtubule-associated protein 1 light chain 3 (a catabolic marker of autophagy) was not different between groups (Figure 1C). Calpain activity was significantly increased by 120% and 147% in HFrEF compared with Con and HfpEF, respectively (Figure 3A). Similarly, ubiquitin proteasome activity in HFrEF was significantly higher by 245% and 155% compared with Con and HfpEF, respectively (Figure 3B).

**Diaphragm**

Although IGF-1 (Figure 2D) and microtubule-associated protein 1 light chain 3 (Figure 2F) did not differ between groups, the protein expression of MuRF1 was significantly increased by 176% and 185% in HFrEF and HfpEF, respectively, compared with Con (Figure 2E). Calpain activity was significantly reduced in HfpEF (Figure 3C), whereas no difference was detected between groups for proteasome activity (Figure 3D).

**Mitochondrial Indices**

**Soleus**

A 47% and 51% significant reduction in the SDH/LDH ratio (an index of oxidative metabolism) was observed in HFrEF compared with that observed in Con and HfpEF, respectively (Figure 4A), whereas no differences were found in creatine kinase activity between groups (Figure 4B). Compared with Con and HfpEF, expression of the mitochondrial transcriptional coactivator PGC-1α was significantly reduced in HFrEF (Figure 5A and 5B), at both the mRNA and protein level by 45% and 31%, respectively, versus Con.

**Diaphragm**

No changes between groups were found in the SDH/LDH ratio (Figure 4C), creatine kinase activity (Figure 4D), or PGC-1α expression (mRNA and protein; Figure 5C and 5D).

**Enzyme Activities of ROS-Modulating Proteins**

**Soleus Muscle**

Compared with Con and HfpEF, the activity of the ROS-generating enzyme NADPH oxidase was significantly increased.
by 73% and 133% in HFrEF, respectively (Figure 6A). Quantification of ROS scavenging enzymes revealed a significant reduction in the activity of glutathione peroxidase by 35% and catalase by 47% in the HFrEF compared with Con, whereas no changes were observed in HFpEF (Figure 6B and 6C). Compared with Con, however, SOD activity was significantly reduced in both HFrEF and HFpEF by 44% and 60%, respectively (Figure 6D).

**Diaphragm**

No significant difference between groups was found in enzyme activities of NADPH oxidase (Figure 6E) or glutathione peroxidase (Figure 6F). Catalase activity, however, was significantly increased by 70% in HFpEF animals compared with Con, with no change observed in HFrEF (Figure 6G). In contrast, both HFrEF and HFpEF groups showed a significant increase of 69% and 78% in SOD activity compared with Con, respectively (Figure 6H).

**Inflammatory Markers**

Plasma concentrations of inflammatory cytokines were different between groups, with TNF-α significantly increased by 53% in HFrEF and 28% in HFpEF when compared with Con (Figure 7A). In contrast, IL-6 was not different between groups (Figure 7B). The cytokines 1 L-1β and IL-12 were significantly increased in HFpEF relative to Con and HFrEF (Figure 7C-D), specifically by 143% and 90% versus Con, respectively. Also a significant increase, but to a lesser extent, was detected between Con and HFrEF (Figure 7C and 7D).

**Discussion**

This study used established animal models to reveal many novel findings in relation to the different molecular and cellular skeletal muscle alterations that exist between HFrEF and HFpEF, which included:

1. Upregulation in markers of muscle atrophy in HFrEF (ie, MuRF1, calpain, and ubiquitin proteosome) but unchanged or lower levels in HFpEF soleus.
2. Increased oxidative stress in HFrEF (ie, higher NADPH oxidase with lower antioxidative enzyme activities) but not in HFpEF soleus.
3. Impaired mitochondrial indices in HFrEF (ie, a lower SDH/LDH ratio and PGC-1α protein expression) but not in HFpEF soleus.
4. Muscle-dependent alterations between HFpEF and HFrEF limited to limb muscle (soleus), with respiratory muscle (diaphragm) remaining largely unaffected.
5. A distinctive circulating inflammatory cytokine response, with increased plasma concentrations of TNF-α in HFrEF but IL-1β and IL-12 in HFpEF.
Overall, therefore, our findings provide initial evidence that skeletal muscle alterations are exacerbated in HFrEF compared with HFpEF, which are mainly isolated to limb (soleus) rather than to respiratory (diaphragm) tissue, and that the different circulating inflammatory cytokines detected between phenotypes may be potentially mediating such effects (as summarized in the Table). As such, our data provide novel insights into the different molecular alterations and potential treatment targets specific to HFpEF and HFrEF.

Skeletal Muscle Alterations in HF

In recent years, it has become evident that HFrEF and HFpEF are 2 different HF entities with different causes yet similar morbidity and mortality outcomes. One hallmark of both entities is exercise intolerance, with impairments to skeletal muscle (both limb and respiratory) playing a key role in exacerbating the symptoms of breathlessness and fatigue. As expected, we found numerous skeletal muscle alterations that were similar between HFrEF and HFpEF (Table), which

![Figure 3](http://circheartfailure.ahajournals.org/)

**Figure 3.** Activity of the calpain (A and C) and ubiquitin proteasome (B and D) systems in soleus and diaphragm tissue in animals exhibiting heart failure with reduced (HFrEF) or with preserved (HFpEF) ejection fraction relative to controls (con). Values are shown as mean ± SEM.

![Figure 4](http://circheartfailure.ahajournals.org/)

**Figure 4.** Ratio of succinate dehydrogenase (SDH)/lactate dehydrogenase (LDH) enzyme activity (A and C) and creatine kinase (CK) enzyme activity (B and D) measured in the soleus and diaphragm from controls (con) and animals exhibiting heart failure with reduced (HFrEF) or with preserved (HFpEF) ejection fraction. Values are shown as mean ± SEM.
included a reduced expression in the anabolic factor IGF-1 and a lower antioxidant enzyme activity of SOD in the soleus, whereas in the diaphragm a greater expression of the key atrophic marker MuRF1.

As mentioned above, most alterations were restricted to limb (soleus) rather than to respiratory (diaphragm) tissue (Table), which is in accordance to previous studies. For example, no changes between groups in the diaphragm were observed in relation to anabolic (IGF-1) or catabolic (calpain and ubiquitin proteasome systems and autophagy) factors, mitochondrial markers, and oxidative stress measures (ie, NADPH oxidase and glutathione peroxidase activity). The main finding in the diaphragm was related to an increased MuRF1 expression in both HF groups, as well as an increased antioxidant enzyme capacity (eg, SOD). The latter is likely explained by the diaphragm being constantly recruited during respiration, which is exacerbated in HF because of breathlessness, which results in a training effect and offsets alterations seen in limb muscle. As such, the remainder of the discussion will focus on our findings from limb (soleus) skeletal muscle.

Divergent Skeletal Muscle Alterations Between HFrEF and HFpEF

Skeletal muscle alterations have important clinical implications as they provide a strong surrogate of functional capacity and prognosis in patients with HF. Importantly, we have confirmed a highly diverse skeletal muscle characterization between HFrEF and HFpEF tissue (as summarized in the Table), which may have important clinical consequences for the future treatment of patients. In HFrEF, for example, we found that markers were increased for both atrophy (ie, MuRF1 and calpain and ubiquitin proteasome activity) and oxidative stress (higher NADPH oxidase but lower radical scavenger enzyme activities), whereas indices of mitochondrial function were further impaired (ie, lower SDH/LDH ratio and PGC-1α expression). These data support previous HFrEF human or animal studies that found an increased expression of atrophy-related proteins, elevated oxidative stress, and impaired mitochondrial respiration. Critically, however, we observed no changes or even a contrasting response in skeletal muscle of HFpEF animals, which included a downregulation in MuRF1 protein expression and unchanged proteolytic activity. This suggests, therefore, that muscle wasting may play a greater role in HFrEF than in HFpEF, which is supported by data on this topic showing a strong link to mortality in patients with HFrEF.

Unfortunately, at present, evidence of skeletal muscle alterations in HFpEF (in both animals and humans) remains limited. The finding that arteriovenous oxygen content difference reserve is an independent predictor of exercise capacity supports the notion that skeletal muscle or microvascular dysfunction is playing a key role in HFpEF, which is supported by patient data showing an increased intramuscular fat deposition, impaired phosphocreatine recovery rates, a fiber-type shift and also a reduced capillary:fiber ratio. Similarly, our group also recently reported numerous skeletal muscle alterations in an animal model of HFpEF, which included fiber atrophy, impaired mitochondrial respiration, a fiber-type shift, but unchanged (or even reduced) ROS and proteolytic-related markers. The present study, therefore, not only confirms previous findings in HFpEF (from patients and animals) but also advances our current understanding of skeletal muscle alterations in terms of anabolic factors, and additional ROS-handling enzyme activities and mitochondrial indices. What still remains unresolved, however, is whether the main factor(s) limiting exercise capacity in both diseases is similar.

Figure 5. mRNA (A and C) and protein (B and D) expressions of PGC-1α in the soleus and diaphragm in control (con) and heart failure with reduced (HFrEF) or with preserved (HFpEF) ejection fraction animals. Values are shown as mean±SEM.
which specifically includes the role of skeletal muscle dysfunction. Briefly, the potential mechanisms that play a dominant role in limiting exercise tolerance between HFpEF and HFrEF can be broadly categorized as central (ie, cardiac output, heart rate and stroke volume) or peripheral (ie, vascular or intramuscular), as recently reviewed.\textsuperscript{19} Our data at least lend support to the argument that intrinsic skeletal muscle impairments may play a greater role in limiting exercise capacity in HFrEF compared with HFpEF. Indeed, other experiments also indicate that greater vascular rather than intramuscular impairments may occur in HFpEF, as a reduction in diffusive oxygen transport was reported to play a greater role in limiting exercise capacity in patients with HFpEF than in those with HFrEF.\textsuperscript{26} Furthermore, it is also known that HFpEF can induce endothelial dysfunction,\textsuperscript{27} with more recent data showing limb blood flow and vasodilation are impaired during exercise.\textsuperscript{28} Overall, therefore, although our data indicate that greater skeletal muscle alterations are induced in HFrEF compared with HFpEF, further investigations are warranted to determine whether this plays a more dominant role in limiting exercise capacity in one rather than in the other disease entity.

**Underlying Mechanisms of Divergent Skeletal Muscle Alterations in HF**

Although it remains unclear what mechanism(s) is responsible for the large variation in skeletal muscle alterations between HFrEF and HFpEF, our data allow us, at least in part, to speculate about some possible key players. One potential mediator may be the transcriptional coactivator PGC-1\textsubscript{α}, which underpins mitochondrial biogenesis and metabolism.\textsuperscript{29} Our

---

**Figure 6.** Enzymatic activity of nicotinamide adenine dinucleotide phosphate [NADPH] oxidase (A and E), glutathione peroxidase (GPX; B and F), catalase (Cat; C and G), and superoxide dismutase (SOD; D and H) in the soleus and diaphragm from control (con) animals and those of heart failure with reduced (HFrEF) or with preserved (HFpEF) ejection fraction. Values are shown as means±SEM.
data revealed that PGC-1α mRNA and protein expression in the soleus was reduced in HFrEF compared with HFpEF and control animals. It is well established that PGC-1α has multiple cellular influences, with it shown to regulate muscle mass by controlling the expression of atrogenes\(^{30,31}\) and oxidative stress.\(^{18}\) As such, PGC-1α may play a key role in the divergent responses of markers of atrophy, oxidative stress, and mitochondrial activity that we observed between HFrEF and HFpEF.

Alternatively, another mechanism that may underpin the distinctive muscle alterations between HFrEF and HFpEF could be circulating inflammatory cytokines, which were differentially expressed between groups. Specifically, plasma concentrations of TNF-α were higher in HFrEF, whereas IL-1β and IL-12 were more increased in HFpEF. Although a systemic inflammation is generally a characteristic of HF, it is well established that specific cytokines have different effects on skeletal muscle.\(^{32}\) Similar to our findings, TNF-α is increased in patients with HFrEF\(^{33-35}\) and is a key factor regulating muscle mass,\(^{39}\) which can activate the expression of atrogenes such as MuRF1 and MafB\(^{37}\) while suppressing anabolic factors such as IGF-1,\(^{38}\) while also further mediating oxidative stress.\(^{39}\) In addition, TNF-α can downregulate PGC-1α expression and thus impair mitochondrial function.\(^{40}\) That we too found such alterations in our HFrEF animals simultaneous with increased plasma TNF-α levels strongly suggests a causal role of this cytokine in mediating skeletal muscle alterations between HF cohorts—a suggestion further supported by our HFpEF animals having unchanged plasma concentrations of TNF-α alongside fewer skeletal muscle alterations.

Nevertheless, a recent notion has proposed that systemic inflammation is the key trigger of HFpEF, mediating endothelial dysfunction and subsequent myocardial hypertrophy and subsequent myocardial hypertrophy and...
stiffening. However, unlike HFrEF, current evidence remains scarce and is conflicting in relation to the role of inflammatory cytokines in HFpEF. For example, a comparison between patients with HFrEF and HFpEF revealed circulating levels of TNF-α were in fact significantly increased in HFpEF but not in HFrEF, without changes in IL-6 between groups. In accordance with that study, we also found that IL-6 concentrations to be unchanged in HFrEF or HFpEF plasma, supporting the suggestion that this cytokine may not play a key role in skeletal muscle alterations induced during advanced HF. However, our finding that IL-1β levels were significantly higher in HFpEF than in HFrEF and control plasma is important, as cell culture experiments have confirmed that IL-1β can reduce myofibrillar content in differentiated myotubes via an increased expression of the atrogenes MuRF1 and MAFbx. This suggests, at least, that IL-1β has the potential to modulate skeletal muscle alterations in HFpEF. In addition, although we also found that IL-12 plasma concentrations to be increased in HFpEF compared with HFrEF and controls, robust evidence on the effects of this cytokine-modulating skeletal muscle remains at present unknown, but clearly further research is warranted. Overall, therefore, that HFpEF tissue clearly documented significantly fewer changes than HFrEF does lend support to the single syndrome notion, which suggests that HF is a continuum with multiple phenotypes between both extremes.

Limitations
Whether skeletal muscle impairments vary between HFrEF and HFpEF because of different causes remains to be determined. Naturally, our findings must, therefore, be viewed with caution in that our HFrEF and HFpEF groups were animals from 2 different strains that underwent 2 different procedures to induce HF (detailed in Methods section of this article). However, as no model is currently established in 1 strain to induce HFrEF and HFpEF, we feel our findings still provide an important contribution to an area where there remains a paucity of data. In addition, the HFpEF rats were significantly older than the HFrEF rats (35 versus 18 weeks), potentially limiting effects seen to age rather than to HF. However, our data revealed greater (rather than fewer) changes in HFpEF than in HFrEF tissue, which provide strong support that age per se did not influence our findings.

Conclusion
We found skeletal muscle alterations to be exacerbated in HFrEF compared with HFpEF, which were mainly isolated to limb (soleus) rather than to respiratory (diaphragm) tissue. That different circulating inflammatory cytokines were also elevated between HFrEF and HFpEF suggest that these could potentially mediate such effects.

Sources of Funding
Dr Bowen is a recipient of a Postdoctoral Research Fellowship from the Alexander von Humboldt Foundation. Support for this study was provided by the European Commission, Framework Program 7, grant number: EU 602405-2. The authors are supported by grants from the K.G. Jebsen Foundation (Drs Wisloff and Rolim) Norwegian Research Council (Dr Wisloff), and the Liaison Committee between the Central Norway Regional Health Authority and the Norwegian University of Science and Technology (Dr Wisloff).

Disclosures
None.

References


Skeletal Muscle Alterations Are Exacerbated in Heart Failure With Reduced Compared With Preserved Ejection Fraction: Mediated by Circulating Cytokines?

Martin Seiler, T. Scott Bowen, Natale Rolim, Maja-Theresa Dieterlen, Sarah Werner, Tomoya Hoshi, Tina Fischer, Norman Mangner, Axel Linke, Gerhard Schuler, Martin Halle, Ulrik Martin Seiler, T. Scott Bowen, Natale Rolim, Maja-Theresa Dieterlen, Sarah Werner, Tomoya Hoshi, Tina Fischer, Norman Mangner, Axel Linke, Gerhard Schuler, Martin Halle, Ulrik

Wisloff and Volker Adams

Circ Heart Fail. 2016;9:
doi: 10.1161/CIRCHEARTFAILURE.116.003027

Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
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/9/9/e003027

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Heart Failure can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Heart Failure is online at:
http://circheartfailure.ahajournals.org//subscriptions/