EDITORIAL COMMENT

Cat-apulting Toward a Molecular Understanding of HFpEF*

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Heart failure with preserved ejection fraction (HFpEF) is marked by increased left ventricular (LV) filling pressure, elevated LV stiffness, and prolonged relaxation in the presence of normal systolic function. Patients with HFpEF often develop pulmonary hypertension with consequent right ventricular (RV) failure, and they present with exercise intolerance associated with skeletal muscle impairment. Although patients with heart failure with reduced ejection fraction (HFrEF) (systolic heart failure) have seen clinical benefits through pharmacologic management over the last >20 years, clinical trials using standard-of-care HFrEF medications have failed to demonstrate significant efficacy in patients with HFpEF. Thus, it is crucial to expand investigative efforts into the molecular mechanisms mediating HFpEF, with the objective of discovering novel therapeutic targets.

Insights into the molecular basis of HFpEF have been provided by RNA sequencing (RNA-seq) analysis of human endomyocardial biopsies [1]. However, because these subjects are not amenable to experimental manipulation, determining whether the observed changes in gene expression contribute to the pathogenesis of HFpEF is a challenge. To circumvent this issue, much work has focused on developing murine models of HFpEF that are amenable to genetic and pharmacologic interventions, and a significantly improved mouse model was recently described [2]. Nonetheless, for translational cardiac research, mice have deficiencies due to their small size and distinct physiology compared with humans.

The Houser group previously described a feline model of HFpEF based on slow, progressive ascending aortic banding (AB) [3,4]. The larger size of the model relative to mice facilitated detailed characterization of cardiopulmonary structure and function, which revealed many features in common with human HFpEF, including LV hypertrophy and fibrosis, elevated LV end-diastolic pressure and left atrial volume, pulmonary hypertension, and evidence of peripheral muscle remodeling. Furthermore, histone deacetylase (HDAC) inhibition was found to be efficacious at attenuating LV stiffness and pulmonary dysfunction in the model [4], establishing it as a viable testbed for assessing novel therapeutic approaches for heart failure, and for molecular dissection of HFpEF pathogenic mechanisms.

In this issue of *JACC: Basic to Translational Science*, Gibb et al [5] comprehensively examined LV and skeletal muscle transcriptional and metabolic changes in this feline model using unbiased multiomics approaches. Importantly, among other things, the data establish that impaired mitochondrial function and an altered metabolic environment in the heart precede cardiac remodeling and dysfunction in HFpEF, suggesting altered cardiometabolic pathways as disease-driving as opposed to consequential, which has remained a difficult-to-test experimental hypothesis. Furthermore, although targeted gene expression and metabolite abundance have been quantified in soleus and vastus lateralis muscle in animal models of HFpEF and in humans with HFpEF, respectively, this is the first study, to our knowledge, to simultaneously quantify transcriptomic and metabolomic alterations in both cardiac and skeletal muscle from individuals with HFpEF.

One month post-AB, cardiac hypertrophy and fibrosis were observed, but without notable changes in heart physiology. Overlaying gene expression with metabolomics data obtained with LV tissue from these animals suggested an early switch from oxidative to glycolytic metabolism, as previously described in HFrEF. Metabolic changes leading to depressed oxidative metabolism in the LV 1 month post-AB included elevated acyl-carnitine and branched chain amino acids, and down-regulation of components of the oxidative phosphorylation pathway. These changes coincided with reduced respiratory rates of electron transport chain complexes I and III in state 3 respiration, described as the ADP-stimulated respiration of isolated coupled mitochondria. Surprisingly, the respiratory rates of electron transport chain complexes reverted back to baseline by 4 months post-AB, and changes in metabolite abundance in the LV were also less pronounced at this later time point. This, coupled with the fact that the down-regulation of expression mitochondrial oxidative machinery seen at 1 month was restored by 4 months post-AB, may reflect adaptation to acute changes in mitochondrial metabolism with pressure overload. Whether mitochondrial respiratory properties would be compromised once again if the pressure-overload stressor persisted beyond 4 months remains to be determined in this model.

Metabolomics analysis suggested a role for enhanced extracellular matrix (ECM) protein synthesis, post-translational modifications, and collagen stabilization in fibrotic remodeling of the heart. Surprisingly, however, RNA-seq analysis failed to reveal dramatic alterations in expression of genes encoding ECM components. It possible that an early burst of cardiac fibroblast proliferation and activation post-AB leads to enhanced ECM production, but that the gene program mediating these events wanes by 1 month post-AB. Furthermore, future single-cell RNA-seq studies in this feline model could unmask profibrotic gene expression programs that were not observed in the current bulk RNA-seq study.

The investigators used a similarly powerful systems biology approach to uncover transcriptomic and metabolomic changes in a peripheral organ, skeletal muscle, coinciding with progression of the HFpEF phenotype. Remarkably, mild transcriptional and metabolic changes were already observed in skeletal muscle 1 month post-AB, preceding any measurable decline cardiac function. Given the use of AB as a "cardiocentric" trigger for HFpEF in the absence of comorbidities such as obesity, these data suggest that

heart-derived factors communicate with skeletal muscle to contribute to impaired physical performance in patients with HFpEF.

At 1 month post-AB, there were 99 and 21 differentially expressed genes in heart and skeletal muscle, respectively, as determined by RNA-seq analysis. In both cases, AB primarily led to down-regulation of gene expression. Given the role of HDACs in transcriptional repression, it is intriguing to speculate that the prior demonstration of efficacy of HDAC inhibition in this feline HFpEF model was due, at least in part, to derepression of gene expression (4). The investigators also noted down-regulation of expression of genes encoding other epigenetic regulatory proteins (eg, BRD4), which could contribute to the observed suppression of gene expression.

The number of differentially regulated genes in skeletal muscle 4 months post-AB were comparable to changes in the heart of the same animals (163 vs 168). The modest number of differentially expressed genes in both LV and skeletal muscle at 1 and 4 months post-AB, with a statistically lenient false discovery rate of 0.1 applied to the current study, is noteworthy. This might be an indicator of pathophysiological heterogeneity in the feline model, which is also observed in the clinic and is thought to contribute to the inability of physicians to effectively treat HFpEF patients. Or, as alluded to in the preceding text, the transcriptomic analyses could be confounded by the kinetics of gene expression changes post-AB, or the use of bulk tissue RNA-sequencing.

Similarly to observations in the heart, overlaying skeletal muscle transcriptomics and metabolomics data suggested an oxidative-to-glycolytic metabolic switch, but unlike in the LV, reduced oxidative phosphorylation respiratory capacity was sustained in skeletal muscle at 4 months post-AB. These data support and emphasize the concept of HFpEF as a systemic multiorgan syndrome, and highlight the role of the heart in mediating deleterious effects in peripheral tissues. Identification of heart-derived circulating factors that govern skeletal muscle dysfunction could lead to novel approaches to treat exercise intolerance in HFpEF.

The combined use of a tractable and translationally relevant animal model with state-of-the art systems biology technologies by Gibb et al (5) sets the stage for follow-up reductionist approaches to validate the roles of specific proteins and metabolites in the control of HFpEF pathogenesis, with the potential of revealing innovative therapeutic targets. Comparing RNA-seq data from the feline model with data obtained using endomyocardial biopsies from HFpEF

patients (1) could narrow the list of potentially translatable targets.

The feline HFpEF model is based on placement of a loose AB around the ascending aorta, into which the animals grow over time, resulting in an insidious decline in diastolic function, with cardiac hypertrophy, fibrosis, and pathological pulmonary remodeling. We view this cardiocentric trigger for HFpEF as an advantage of the model, because it enables molecular dissection of heart-driven mechanisms for cardiac and systemic dysfunction in HFpEF. Nonetheless, because HFpEF patients are often obese and/or have diabetes, hypertension, and advanced age, future studies should incorporate 1 or more of these comorbidities into the model, as was recently described in mice and pigs (2,6). Furthermore, in human HFpEF cohorts, females consistently outnumber males by ~3:1, with sex-specific differences in clinical presentation, such as worse ventricular systolic reserve during exercise in females compared with males. Thus, it is essential to assess the impact of progressive pressure overload in female cats. Given the devastating consequences of RV failure in the context of HFpEF, and the absence of right-sided dysfunction in most preclinical models of diastolic dysfunction/HFpEF, the feline model should also be employed to address mechanisms and interventions for pathological RV remodeling.

This seminal work of Gibb et al (5) provides a foundation to address many important issues on the pathophysiology of HFpEF, a form of heart failure that affects millions of individuals worldwide, and for which no approved therapies exist. Interrogation of existing and forthcoming data obtained with the feline HFpEF model will unquestionably catapult the field forward.

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