HIGHLIGHTED TOPIC | Exploring New Concepts in the Management of Heart Failure with Preserved Ejection Fraction: Is Exercise the Key for Improving Treatment?

Cell- and molecular-level mechanisms contributing to diastolic dysfunction in HFpEF

© Kenneth S. Campbell¹ and Vincent L. Sorrell²

¹Department of Physiology and Center for Muscle Biology, Linda and Jack Gill Heart Institute, University of Kentucky, Lexington, Kentucky; and ²Division of Cardiovascular Medicine, Linda and Jack Gill Heart Institute, University of Kentucky, Lexington, Kentucky

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Campbell KS, Sorrell VL. Cell- and molecular-level mechanisms contributing to diastolic dysfunction in HFpEF. J Appl Physiol 119: 1228–1232, 2015. First published April 24, 2015; doi:10.1152/japplphysiol.01168.2014.—Heart failure with preserved ejection fraction (HFpEF) is the default diagnosis for patients who have symptoms of heart failure, an ejection fraction >0.5, and evidence of diastolic dysfunction. The clinical condition, which was largely unrecognized 30 years ago, is now a major health problem and currently accounts for 50% of all patients with heart failure. Clinical studies show that patients with HFpEF exhibit increased passive stiffness of the ventricles and a slower rate of pressure decline during diastole. This review discusses some of the cell- and molecular-level mechanisms that contribute to these effects and focuses on data obtained using human samples. Collagen cross-linking, modulation of protein kinase G-related pathways, Ca²⁺ handling, and strain-dependent detachment of cross bridges are highlighted as potential factors that could be modulated to improve ventricular function in patients with HFpEF.

heart failure; myocardial stiffness; myocardium; myocyte; ventricular function

HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFpEF) already accounts for ~50% of all patients with heart failure and is becoming steadily more common (37). The trend (~60,000 new patients with HFpEF per year in the United States) (18) probably reflects the increasing incidence of risk factors for HFpEF, which include obesity, diabetes, and hypertension (31), and the dramatic increase in the number of older people. For example, in the United States, the number of people over 85 will increase by 350% between 2000 and 2050 (48). Because no treatments have yet been shown to improve outcomes for patients with HFpEF (5), the condition has become a major health problem and is likely to become even more significant in the coming years. New treatment strategies are required and would have a major clinical impact.

Early research efforts focusing on HFpEF were hampered by disagreements about how to define the condition. Progress has been made in this area, and four sets of guidelines now agree that formal diagnosis of HFpEF requires symptoms of heart failure, evidence of normal systolic left ventricular function, and indications of abnormal diastolic function (1, 38, 46, 50). There is also consensus that the symptoms of patients who have HFpEF become worse when they exercise. What is not yet clear is why this occurs and what clinicians can do to help their patients.

HFpEF is a complex condition, and numerous factors, including but not limited to pulmonary vascular disease, vascular stiffening, and autonomic dysfunction are likely to contribute to clinical symptoms (5). Some of these topics are considered elsewhere in this review series. This article focuses on cell- and molecular-level mechanisms that are specific to the heart. The main emphasis is on factors that influence how quickly the myocardium relaxes and how stiff the myocardium is during diastole. In addition, this review suggests several therapeutic strategies that could potentially be employed to improve ventricular filling. If any of these can be developed into a useful treatment, it might offer new hope for patients afflicted by the condition.

Ventricular Function in Patients with HFpEF

By definition, patients with HFpEF have “preserved” left ventricular global systolic function, as measured by the left ventricular ejection fraction (LVEF). Indeed, meta-analysis
shows that HFpEF increases LVEF above the values measured in control groups (17). Imaging-based studies also show that HFpEF does not reduce left ventricular end-diastolic volume (5) and may actually increase chamber size (35), although the effect is controversial (54). Together, these data imply that dyspnea in patients with HFpEF, as in heart failure with reduced ejection fraction, is most likely to result from elevated filling pressures. That is, the ventricles fill to their normal size but require more pressure to do so.

This reasoning has now been confirmed in numerous studies. In HFpEF, the diastolic pressure-volume relationship is elevated, and the rate at which pressure declines after the aortic valve closes is reduced (47, 53). These organ-level effects correspond to higher and steeper passive force/length curves and slow force relaxation at the tissue (myocardial) level. Figure 1 summarizes these effects in schematic form.

This type of presentation suggests that HFpEF produces two separate mechanical effects. The elevated force/length curve implies that HFpEF increases the passive stiffness of myocardial tissue (that is, the static force at a given length). The slow relaxation suggests that HFpEF is modulating a time-dependent property (that is, how quickly force is dropping). Although this distinction may be simplistic, it provides a convenient way of describing the cellular- and molecular-level effects that are likely to be important in HFpEF (Table 1).

**Myocardial Stiffness**

Early experimental work by Granzier and Irving (19) showed that there are three main sources of passive stiffness in myocardium: the collagen-based extracellular matrix, titin molecules, and intermediate filaments. Collagen dominates myocardial stiffness at very long sarcomere lengths, whereas titin seems to be the most important factor for cells operating in their normal working range (sarcomere lengths of ~1.8 to ~2.2 μm). Granzier and Irving (19) calculated that intermediate filaments only contribute ~10% of myocardial stiffness, and accordingly they have not been studied extensively in the context of heart failure. Collagen and titin, in contrast, have been studied in considerable detail.

The stiffness of the collagen matrix depends on the amount of collagen and the extent of collagen cross linking. Regulation of collagen content is exceptionally complicated and is probably a very dynamic process (32). Some estimates suggest that myocardial collagen is completely replaced every ~100 days (33). Synthesis ultimately depends on the expression of collagen-type-specific genes, whereas breakdown is governed by the amount and activity of matrix metalloproteinases. These proteinases are in turn regulated by tissue inhibitors of matrix metalloproteinases. Many details of the biochemical pathways are clinically significant, and several collagen-related proteins are being studied as potential biomarkers. For example, procollagen type 1 carboxy-terminal propeptide is released into the serum and can be used to estimate the rate of collagen synthesis in the heart (39).

Measuring the extent of collagen cross linking in human patients is more difficult because it requires collecting a sample of myocardial tissue. Nevertheless, López et al. (34) were able to show that collagen cross linking is related to elevated filling pressures in patients with heart failure. These authors also identified lysyl oxidase, an enzyme that regulates cross linking, as a potential mediator of the effect. These are particularly interesting data because it is known that some of the risk factors for HFpEF (for example, hypertension, advanced age, and diabetes) increase cross linking (52).

López at al. (34) also measured collagen content in their study, but this value did not predict left ventricular filling pressures (34). One interpretation of these data is that it is the extent of collagen cross linking rather than the total amount of collagen that influences ventricular function. This could also explain why two-thirds of biopsies from patients with HFpEF do not have elevated collagen volume fractions (4). It is also possible that chamber stiffness is affected by diffuse fibrosis, which is harder to quantify in an unambiguous way. Clearly, more translational studies are required.

Titin molecules are huge proteins (molecular weight ~3 MDa) that link the thick filaments of sarcomeres to their adjacent Z-disks. Healthy humans express two isoforms, N2B and N2BA, in an ~65:35 ratio (30), but HFpEF has been shown to increase the relative proportion of the N2BA isoform (4). Because the N2BA isoform is larger (and thus less extended at a given sarcomere length) than its N2B counterpart, the isoform switch would be expected to decrease passive tension (49). However, this contradicts organ-level measurements, which clearly show that HFpEF is associated with increased rather than decreased ventricular stiffness. As a result, isoform switching of titin has been viewed to date primarily as a compensatory rather than a causative effect of ventricular dysfunction in HFpEF (44).

The molecular mechanics of titin can also be influenced by posttranslational modifications (24). Protein kinase C (PKC) is activated by the α1-adrenergic system and increases the passive stiffness of titin (25). In contrast, PKA (29) and PKG (28) decrease the stiffness of titin. PKA modulates many processes in the heart, but it might be possible to develop a relatively specific treatment for HFpEF by manipulating PKG-related mechanisms.

Data supporting this hypothesis include the work of van Heerebeek et al. (45), who demonstrated that in vitro treatment...
with PKG reduces passive force in myocytes isolated from patients with HFpEF. Sildenafil, which increases myocardial PKG activity by inhibiting phosphodiesterase type 5A, had also been shown to improve diastolic function in patients with HFpEF with pulmonary hypertension (20). Unfortunately, these early results were not duplicated in the larger RELAX trial, which tested the effects of 24 wk of sildenafil treatment in 214 patients (40). This important trial showed that sildenafil did not impact the clinical status or exercise capacity of patients with HFpEF, which obviously lowers the probability of sildenafil becoming a widely implemented therapy for HFpEF. It remains possible, however, that targeting other steps in the PKG pathway will prove beneficial. For example, inhibition of phosphodiesterase 1 (which is expressed more highly in heart than phosphodiesterase 5A) reduced isoproterenol-induced hypertrophy in mice (36). Other potential approaches include increasing the synthesis of cGMP using nitric oxide donors or natriuretic peptides. Tachyphylaxis (reduced re-transients in future experiments as well as protein levels will help to advance the field, but the functional measurements will present logistical difficulties to most research groups. This is because measuring Ca\textsuperscript{2+} transients requires fresh tissue samples as opposed to the previously frozen samples that are often used in contraction assays. Animal-based studies are therefore particularly important for this area of research.

Hiemstra et al. (26) studied myocytes isolated from a mini swine model of HFpEF and showed that this type of heart failure reduced the amplitude of Ca\textsuperscript{2+} transients and slowed Ca\textsuperscript{2+} reuptake. These data support the hypothesis that impaired Ca\textsuperscript{2+} handling may contribute to slow relaxation in HFpEF. Because Hiemstra et al. (26) did not present data from mini swine with heart failure and reduced ejection fraction, it is difficult to compare their data to the human results of Hamdani et al. (22, 23).

Clearly, there is an exciting opportunity here. The first experiments that simultaneously compare Ca\textsuperscript{2+} handling in patients or animals that 1) do not have heart failure, 2) have HFpEF, and 3) have heart failure with reduced ejection fraction, will have a significant impact on the field.

The final mechanism that will be discussed in this review is strain-rate-dependent detachment of cross bridges. Chung and Campbell (14) have recently extended pioneering work started by Brutseart and his colleagues in the 1970s and 1980s (7) by studying how quickly trabeculae relax after loaded twitch contractions. Brutseart had shown that myocardium relaxed faster when it contracted against a low afterload (6) but was not able to control for a potential confounding effect of tissue strain rate. Chung and Campbell used a revised protocol and separated the effects of afterload and re-lengthening. Measurements performed using murine, rat, and human trabeculae showed that the rate of relaxation increased...
linearly with the tissue strain rate at end systole but was not independently modulated by afterload. Subsequent computer modeling (11) reproduces this behavior and suggests that quick stretches speed myocardial relaxation by detaching myosin heads and thereby disrupting cooperative mechanisms that would otherwise prolong thin filament activation. Modulating cooperativity (for example, by increasing the stiffness of tropomyosin molecules) so that unactivated sites on actin increase the rate at which other sites turn off could therefore alter relaxation kinetics as well as the time course of force development (9).

Although Chung and Campbell’s (14) data were collected using trabeculae, the same mechanisms may influence ventricular-level relaxation as well. Rosen et al. (41) used high-speed MRI to show that the heart reverses its systolic motion, releasing torsion and shear before the aortic valve opens. This indicates that portions of the myocardium are relengthening before relaxation. It is not yet clear whether these nonuniformities reflect recoil against the extracellular matrix, transmural heterogeneity of action potential duration (2), or other mechanisms. However, echocardiography clearly demonstrates that rapid untwisting of the heart modulates early ventricular filling (8).

Together, these data suggest two strategies that could potentially be developed into useful treatments for HFpEF. The first is to modulate the contractile properties of the myofilament system. This could be achieved by using viral technologies to alter the expression profile or posttranslational status of sarcomeric regulatory proteins or by using small molecules to alter the kinetics of specific cross-bridge transitions (16). A second potential strategy is to modulate end-systolic strain rate in different regions of the heart. This could be implemented using novel pacing methods and could be tested immediately in large animal models of HFpEF. At least in theory, it might also be possible to test the hypothesis in carefully controlled studies with human patients when a biventricular intracardiac device is being implanted as part of normal care.

Summary

HFpEF is a serious condition that is impacting a steadily increasing number of patients. To date, no treatments have been shown to improve clinical outcomes for HFpEF, and the molecular mechanisms that underlie the disease are not yet clear. New strategies that could be tested include targeting collagen cross linking, PKG-related pathways, calcium-handling mechanisms, myofilament cooperativity, and strain rate-dependent detachment of cross bridges.

REFERENCES

22. Hamdani N, Bishu KG, von Frielings-Salewsky M, Redfield MM, Linke WA. Deranged myocardial phosphorylation and function in ex-


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