Animal Models of Heart Failure

A Scientific Statement From the American Heart Association

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Heart failure (HF) is a leading cause of morbidity and mortality in the United States. Despite a number of important therapeutic advances for the treatment of symptomatic HF, the prevalence, mortality, and cost associated with HF continue to grow in the United States and other developed countries. Given the aging of our population and the prevalence of diseases such as diabetes mellitus and hypertension that predispose patients to this syndrome, it is possible that HF prevalence will increase in the next decade. Current treatments primarily slow the progression of this syndrome, and there is a need to develop novel preventative and reparative therapies. Development of these novel HF therapies requires testing of the putative therapeutic strategies in appropriate HF animal models.

The purposes of this scientific statement are to define the distinctive clinical features of the major causes of HF in humans and to recommend those distinctive pathological features of HF in humans that should be present in an animal model being used to identify fundamental causes of HF or to test preventative or reparative therapies that could reduce HF morbidity and mortality.

HF is a clinical syndrome with primary symptoms including dyspnea, fatigue, exercise intolerance, and retention of fluid in the lungs and peripheral tissues. The causes of HF are myriad, but the common fundamental defect is a decreased ability of the heart to provide sufficient cardiac output to support the normal functions of the tissues because of impaired filling and/or ejection of blood.

HF is a significant health burden in both the developed world and in emerging nations. In the United States, over a half million new diagnoses of HF occur each year, and the prevalence is 5.8 million individuals >20 years of age. HF has a substantial societal burden, with yearly costs in the United States estimated to be $39.2 billion. The increasing prevalence of HF is due in part to the aging of the population, but prevalence of HF is also increasing because better treatment and increased survival of ischemic cardiac disease earlier in life result in survivors at risk for HF in the longer term. HF is recognized as a progressive syndrome, and in 2005 the joint American College of Cardiology/American Heart Association guidelines proposed a new classification of HF based on the recognition of 2 stages preceding symptomatic HF (Figure 1) and symptomatic (stage C) and refractory (stage D) symptomatic HF, as well.3,4 This scheme is a conceptual framework not meant to displace the well-established New York Heart Association classification scheme that defines progressive clinical symptoms and signs of HF. The American College of Cardiology/American Heart Association schema and New York Heart Association classifications should be used by HF investigators to inform assessment and classification of animal models.

Although the current standard of care for HF improves outcomes, the syndrome continues to progress and there is a need for novel therapies that can prevent, further slow the progression, and/or reverse the structural and functional defects of the failing heart. Research to identify novel targets requires testing of the putative therapeutic strategies in appropriate HF animal models.
for HF therapy usually requires preclinical testing in appropriate HF animal models. Although numerous animal models are available for use, there are inadequate standards for what clinical features should be present in these models, and the presence or absence of the HF phenotype is often not documented.

The intention of this statement is to define critical features of HF and propose a set of parameters that investigators should measure to ensure that they have an animal model with the clinical features known to be present in HF patients. The statement discusses the critical features that are present in patients with HF induced by specific causes and discusses animal models that mimic these clinical scenarios. The statement seeks to identify standard features of HF in the whole animal (increased activity of the sympathetic nervous system is an example), within the heart (increased filling pressures is an example), and at the cellular level (expression of fetal genes is an example). The statement will review approaches for producing HF animal models with critical features of HF in humans will have a higher likelihood of translating to HF patients.

It is understood that HF in humans is a complex clinical syndrome that can be caused by a variety of diseases. In the clinical realm, chronic hypertension and ischemic heart disease are major contributing factors. In addition, many forms of acquired, structural, and genetically determined disorders can underlie the clinical presentation. In some cases, animal models may mimic the human condition closely. In others, an acute intervention such as coronary obstruction may mimic only a single discrete time point of an otherwise chronic disease that develops over a lifetime. In addition, animal models are often developed on a defined genetic background that does not reflect the diversity of human populations, which can result in a variety of phenotypes from the same monogenic disorder. Despite these limitations, properly assessed animal models have much to offer to the advancement of clinical care. Investigation of molecular pathways in early or late stages of HF can identify novel targets for therapeutic intervention or biomarkers for disease progression. Studies in large-animal models usually provide important preclinical proof of concept for novel therapies before US Food and Drug Administration-approved clinical trials. Helping investigators develop well-characterized HF animal models with characteristics that reproduce key

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**Figure 1.** Stages in the development of heart failure/recommended therapy by stage. FHx CM indicates family history of cardiomyopathy; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; HF, heart failure; MI, myocardial infarction; LV, left ventricle; LVH, left ventricular hypertrophy; EF, ejection fraction. Reprinted with permission from Hunt et al.©2005 American Heart Association.
Valvular Lesions That Cause HF

Description of Overall Clinical Entity

The canonical symptoms of HF, which include shortness of breath, peripheral and pulmonary edema, and low exercise tolerance, can arise from structural defects in the aortic and/or mitral valve. The valvular lesions that necessitate medical and surgical interventions include those which are due to stenosis (abnormally high resistance to ejection and failure to fully open) or regurgitation (a failure of complete coaptation of the leaflets and adequate closure). For the purposes of illustration and focus, a prototypical lesion such as aortic stenosis (which causes a significant left ventricular (LV) pressure overload) and that of mitral regurgitation (which causes a significant LV volume overload) will be discussed with respect to the pathophysiology and natural history of events that ultimately lead to HF. Although each of these lesions can result in increased LV diastolic/atrial pressures causing fluid retention and fatigue, the underlying pathophysiology of aortic stenosis (AS) and mitral regurgitation (MR) are quite distinct.

Causes and Associated Features of AS

Common causes of AS include atherosclerotic disease with or without calcification, calcification independent of atherosclerosis, and aortic valve malformations (ie, bicuspid aortic valve). All result in increased stiffness of the aortic valve and reduce orifice area. The increased resistance to LV ejection with AS causes increased LV afterload. The physical obstruction to LV ejection requires increased pressure to be developed to propel blood across the reduced aortic orifice. Under normal conditions, the resistance to ejection offered by the open aortic value is very small, and there is no perceptible pressure gradient across the valve during ejection. AS causes a higher than normal resistance to ejection, and increased LV pressure is required throughout the ejection phase to eject the normal stroke volume. As a consequence, a difference between the LV and aortic pressures occur during the ejection phase, which is defined as the LV-aortic pressure gradient. The magnitude, duration, and progression of this pressure gradient are the determinants that stimulate the myocardial response. Specifically with AS, significantly increased LV systolic wall stress occurs and thereby evokes myocardial growth, LV hypertrophy (LVH). In AS, LVH is characterized as concentric hypertrophy whereby wall thickness is increased while LV volumes remain the same or decrease. At the cellular level, myocytes undergo hypertrophy by adding sarcomeres in parallel to achieve an increase in width. In addition, fibroblasts proliferate within the myocardium and in concert with localized activation of a number of bioactive molecules, resulting in increased extracellular matrix deposition. Structural hallmarks of prolonged AS are significantly increased collagen accumulation between individual hypertrophied myocytes and myocyte fascicles. In the most common forms of AS, there is an initial “compensatory” phase in which indices of LV pump function such as ejection fraction are within normal limits. However, this phase is associated with increased myocyte cross-sectional area and progressive accumulation of myocardial extracellular proteins and fibrosis. Thus, LV active relaxation, which depends on myocyte Ca2+ resquestration, and passive relaxation, which depends on myocardial stiffness, become abnormal. In particular, enhanced synthesis and deposition of myocardial matrix is directly associated with increased LV myocardial stiffness, which causes disturbed filling characteristics during diastole. Clinical studies of patients with AS and significant LVH suggest that a fundamental structural milestone in the transition from this compensated state to HF symptoms is myocardial fibrosis with diastolic dysfunction. The progressive impairment in LV diastolic function with AS results in elevated LV diastolic and left atrial pressures, atrial enlargement, increased pulmonary venous pressures, and subsequently the manifestation of HF symptoms. In patients with AS, the development of systolic dysfunction such as a fall in LV ejection fraction, and diastolic dysfunction, as well, is an extremely poor prognostic sign and represents a “decompensated” condition. Although the relief of AS can be achieved through aortic valve replacement and results in significant regression of LVH, abnormalities in myocardial extracellular matrix content persist for months to years. Thus, in clinical AS there is a compensated phase with LVH and relatively normal LV systolic function. Later in time, with increases in myocardial fibrosis, there is diastolic dysfunction and, eventually, decompensation with pump failure and a poor prognosis.

Critical Features of an Animal Model of AS in Humans

A critical and poorly reversible feature of progressive LVH and subsequent HF is myocardial fibrosis with diastolic dysfunction. As such, critical features of an animal model that would replicate human AS would include the following:

1. A slowly evolving LV-aortic pressure gradient (Figure 2),
2. Initial development of LVH with increased myocyte cross-sectional area, myocardial fibrosis, and normal ejection fraction,
3. Progression of myocardial fibrosis and diastolic dysfunction resulting in increased filling pressures that lead to left atrial enlargement and eventually reduced systolic function with the development of HF symptoms.

Large-Animal Models of AS

Large animal models with progressive aortic constriction within the supravalvular position have been described in cats, dogs, sheep, and pigs. These animal models replicate many of the critical features of human AS including progressive increases in the LV-aortic pressure gradients and a compensatory LV remodeling response, significant LVH with myo-
Overall, this progressive increase in LV-aortic pressure gradient and LV mass, whereby a significant gradient and nearly doubling of LV mass occurs after 2 months of incremental increases in LV afterload. Although significant LVH was achieved, an acute decompensation in LV ejection fraction did not occur. LV indicates left ventricle; Ao, aortic; LVH, left ventricular hypertrophy. Adapted from Tagawa et al. 

Small-Animal Models of AS
The most common model of AS in small animals is transverse aortic constriction (TAC) in the mouse. This technique causes a fixed aortic constriction and an abrupt increase in LV afterload, and can cause such severe constriction that there is acute hemodynamic instability with reduction in ejection fraction (EF) and early postoperative mortality. 

The relative degree of mortality and immediate decline in LVEF can be attenuated to some degree by reducing the severity of the TAC. The inciting stimulus for LVH produced by acute, severe pressure overload is likely to be different than in animal models with slow progressive pressure overload and in patients with AS. Therefore, activation of growth regulatory pathways and contractile and Ca²⁺ regulatory proteins and extracellular remodeling, as well, may have less relevance to humans with AS. In addition, the myocardial fibrosis and diastolic dysfunction that develop in these models could represent a primary defect in LV systolic dysfunction or could be secondary to the acute cardiac decompensation that is often present in this model. The utility of mouse models is the ability to test the roles of specific molecules in TAC-induced cardiac dysfunction in genetically modified mice. The weaknesses of these models include the fact that they do not have some of the key features of the disease in humans, including the inability to easily induce slow progressive pressure overload. Therefore, an integrated approach that identifies and tests putative AS HF targets in mouse models and then validates these targets in an appropriate large-animal model could provide a solid platform to develop new AS therapies.

Causes and Critical Features of MR
The mitral valve apparatus contains the mitral leaflets, mitral annulus, chordae tendineae, and papillary muscles. Cardiovascular diseases that affect one or all of these structures can result in significant mitral valve incompetence (allowing the retrograde flow of blood from the ventricle to the atria during systole [MR]). MR is the most common valvular disorder and can arise from mitral valve prolapse, papillary muscle dysfunction secondary to ischemic heart disease, endocarditis, and rheumatic disease. LV dilation from ischemic or cardiomyopathic disease can also cause MR. The LV loading abnormality with MR is diastolic volume overload. During LV systole, which includes the isovolumetric contraction and the ejection phases, as well, the pressure developed within the LV first causes retrograde ejection of blood into the left atrium through the incompetent mitral valve. Thus, there are 2 pathways for LV ejection: a low-impedance path through the mitral valve and into the left atrium and a higher-impedance path through the aortic valve. As a consequence, abnormally high LV emptying occurs during systole and results in low LV end-systolic volumes. The total LV stroke volume is therefore divided between the regurgitant volume (into the atria) and volume ejected through the aorta (the forward stroke volume). A common calculation in MR is the regurgitant fraction, which is the ratio of the regurgitant volume and total stroke volume expressed as a percentage. The severity of MR is often quantified by the regurgitant fraction, and this parameter is used as an index for the likelihood for progressive LV myocardial remodeling, dysfunction, and eventual HF. During the compensated (pre-HF) phase of MR, adequate forward stroke volume into the aorta is maintained by augmenting LV end-diastolic volume and total stroke volume. A unique hemodynamic feature of compensated MR is that LVEF is supranormal because of the low-impedance ejection pathway, and this makes the assessment of LV muscle contractility difficult. In chronic and severe MR, LV dilation continues, with progressive enlargement of the left atrium and increased pulmonary venous pressures with signs and symptoms of HF. If this disease progresses without correction, then LV myocardial contractile dysfunction occurs with a rapid decline in hemodynamic status and HF. 

The fundamental mechanical driving force for changes in LV geometry and structure with MR is a chronic and often a progressively increasing volume overload. LV end-diastolic volumes are significantly increased, which results in increased end-diastolic and systolic wall stress with a unique
pattern of eccentric LVH. The myocyte remodeling with MR is the addition of sarcomeres in series to achieve an increase in myocyte length with no significant increase in cross-sectional area. In addition, with significant MR and subsequent LV dilation, a distinctive loss of the collagen fibrils surrounding individual myocytes occurs. This cellular and extracellular remodeling produces a highly compliant LV.

Critical Features of the Animal Model
Critical features to consider in animal models that would replicate the pathophysiologic features of MR would include the following:

1. LV volume overload with significant increases in end-diastolic volume and LV and LA dilation,
2. A supernormal EF with ejection divided between retrograde flow into the atria and antegrade flow through the aortic valve,
3. Eccentric LVH with myocyte lengthening and a disruption/loss of myocardial matrix.

Large-Animal Models of MR
The clinical phenotype of chronic MR can be induced by severing the chordae tendineae, which induces significant MR.17-19 The canine model of MR causes LV dilation and an eccentric LVH pattern, which is accompanied by myocyte lengthening.18,19 Unlike the LVH that occurs in large-animal models of AS, chronic MR in dogs causes severe LV contractile dysfunction at both the chamber and myocyte level. In this chronic MR model, significant myocardial matrix accumulation does not occur, but, instead, histological assessment reveals collagen matrix disruption, again, significantly different than that of LV pressure overload. This chronic MR model has been successfully used to examine the contributory effects of the β-adrenergic and the angiotensin II receptor pathways in the progression of HF in this large-animal model of MR.19,20 These large-animal models of MR replicate some critical features of this form of HF.

LV Volume Overload in Smaller Animals
MR induction in rodents has not been accomplished to date. However, the induction of LV volume overload either through the creation of aortic insufficiency or an aortocaval fistula has been described.21-26 A retrograde catheter technique has been used in rabbits to induce damage to the aortic valve with significant aortic regurgitation and thereby LV volume overload. In this model of LV volume overload, LV dilation and eccentric LVH occurs over a period of weeks to months with accompanying increased LV filling pressures and the manifestations of HF.21,22

LV volume overload can also be induced by creation of a small bridge between the abdominal aorta and inferior vena cava, thereby inducing a functional aortocaval fistula.21-26 Detailed LV morphometric studies have been performed in the rat model of aortocaval fistula and have provided some of the early insight into the level of LV myocardial and myocyte remodeling that occurs with chronic volume overload.23,24 This rat model of LV volume overload has been successfully used to examine the effects of pharmacological interruption of signaling and proteolytic pathways that likely contribute to LV remodeling and failure secondary to a volume overload.25,26 Although this aortocaval fistula model is not caused by valvular defects, this type of LV volume overload replicates many critical features of MR-induced LV remodeling and failure.

Recommendations
Animal models that replicate phenotypic features of AS (pressure overload, concentric hypertrophy, increased myocyte width with no major change in length) and MR (volume overload, eccentric hypertrophy and increased myocyte length with no major increase in myocyte width) in humans are available and provide a valuable resource for identification of novel therapeutic targets and testing novel approaches to improve cardiac structure and function. Critical features of AS animal models are an adaptive phase of concentric LVH with minimal or no chamber dilation. This compensated phase should be followed by fibrosis, diastolic dysfunction, and eventually decompensated systolic failure. Achieving all of these features in a progressive manner in models of AS can be difficult, especially in rodent models. Therefore, putative targets identified in rodent TAC models should be validated in AS animal models with slow progressive pressure overload.

Acute MR animal models should produce rapid and robust changes in LV volumes and geometry, with progressive myocyte lengthening, and a loss of myocardial matrix support. These critical phenotypic geometric and structural hallmarks can be found in large-animal models of MR and in smaller animal models of volume overload.

Critical unresolved issues in patients with valve disease are how to enhance cardiac repair after correction of the valve defects. Animal models that replicate human phenotypes that are amenable to correction of the inciting defects (unbanding of the aorta, repair of the mitral or aortic valve, and fistula correction) would be useful to define better strategies to enhance beneficial cardiac remodeling after repairing those defects that produce pressure and volume overload.

Dilated Cardiomyopathy
Description of the Clinical Entity
Dilated cardiomyopathy (DCM) is characterized by ventricular dilatation, systolic dysfunction (reduced ventricular EF), abnormalities of diastolic filling, and either normal or reduced wall thickness (ie, pathological ventricular remodeling; eccentric hypertrophy). Both diastolic and systolic wall stress are increased in proportion to the HF syndrome. There is biventricular and bialtrial enlargement, elevation of left- and right-sided filling pressures, and an increase in organ and chamber weight with myocyte hypertrophy.27,28 Along with myocardial changes, DCM is also characterized by annular dilatation of the mitral and tricuspid valves, apical displacement of the papillary muscles, and lengthening of the mitral leaflets, and atrioventricular valve regurgitation.29,30 Ventricular remodeling is triggered by index insults (below) and is perpetuated over the long term by factors that include augmented diastolic and systolic wall stress, and the activation of neurohormonal systems not only help to maintain cardiac output, but also to impart deleterious effects in the heart.31-33 The syndrome of HF occurs when the dysfunctional heart cannot maintain adequate output to the peripheral
tissues or can do so only at elevated filling pressures.\textsuperscript{3,34} This results in the classical signs and symptoms of HF that reflect low cardiac output and pulmonary and/or systemic congestion and include fatigue, effort intolerance, exertional dyspnea, fluid retention, and reduced tissue perfusion.\textsuperscript{32,35}

**Causes and Associated Features**

The DCM phenotype results from a broad variety of primary and secondary etiologies. Primary conditions solely affect the heart muscle (idiopathic DCM) and are linked to heterogeneous genetic mutations in cytoskeletal, sarcomemal, sarcomer, and nuclear envelope proteins.\textsuperscript{36} Secondary causes are extensive, with the most frequently encountered clinical conditions being coronary artery disease and antecedent myocardial infarction (ischemic cardiomyopathy) and longstanding hypertension.\textsuperscript{3,37} Other causes include myocarditis (especially viral), Chagas disease, chemotherapy drugs (eg, anthracyclines), sustained and inappropriate tachycardia, autoimmune disorders (eg, systemic lupus erythematosus), endocrine disorders (eg, hypothyroidism, diabetes mellitus), excessive alcohol consumption, nutritional deficiencies, neuromuscular disorders, and peripartum cardiomyopathy.\textsuperscript{36} Despite the diverse array of underlying causes, there are striking similarities in the associated structural, functional, biochemical, and molecular phenotypes\textsuperscript{31,32,35} related to the long-term cardiotoxic effects of augmented mechanical load (increased wall stress) and neurohormonal activation.\textsuperscript{3}

Neurohormonal systems activated in HF include the adrenergic and renin-angiotensin-aldosterone systems, endothelin, vasopressin, and inflammatory mediators.\textsuperscript{3,31–33,35,38,39} Although these systems impart some compensatory effects, their activation over the long term are felt to impart detrimental biological effects that promote adverse remodeling. There is also the elaboration of antihypertrophic factors such as natriuretic peptides, including atrial natriuretic factor and B-type natriuretic peptide, in response to atrial and ventricular stretch.\textsuperscript{40,41} Molecular hallmarks of DCM include activation of the fetal/hypertrophic gene program,\textsuperscript{33,42,43} local and systemic inflammation,\textsuperscript{44–46} and oxidative stress.\textsuperscript{47–49} Common molecular changes include upregulation of atrial natriuretic factor and downregulation of sarcomplasmic reticulum calcium ATPase, α-myosin heavy chain, and β₁-adrenergic receptors.\textsuperscript{43}

The histopathologic hallmarks of DCM are myocyte hypertrophy (increases in myocyte length and width), interstitial and replacement fibrosis, and alterations of the extracellular matrix, progressive cardiomyocyte death (from apoptosis, necrosis, and autophagy), and relative capillary rarefaction.\textsuperscript{32,33,38,50–55} Alterations in ventricular performance result from deranged systolic and diastolic function at rest and diminished contractile reserve on stress, and from persistent and progressive increases in systolic wall stress, as well. DCM hearts exhibit depressed isovolumic (eg, peak dP/dt), ejection phase (eg, EF), and pressure-volume plane indexes (eg, end-systolic elastance), and slower relaxation rates (eg, tau).\textsuperscript{56–59} There is blunted contractile augmentation with catecholamine stimulation and during exercise (β-adrenergic hyporesponsiveness),\textsuperscript{60–64} depression of the stretch-induced force response,\textsuperscript{57,58,65} and blunting of force-frequency responses.\textsuperscript{66–68} At the myocyte level, mechanical dysfunction is a manifestation of altered Ca\textsuperscript{2+} uptake, storage, and release,\textsuperscript{69,70} altered β-adrenergic receptor (β-AR) function (reduced β-AR density and β-AR uncoupling)\textsuperscript{62,63} and activation of CaMKII signaling cascades.\textsuperscript{71}

**Critical Features of the Animal Model**

DCM animal models should reproducibly exhibit the chamber level structural phenotype in humans: spherical LV dilatation, eccentric hypertrophy with relative wall thinning (reduced mass-to-volume ratio), depressed LV systolic and diastolic performance, and reduced functional reserve with provocation (eg, exercise or tachycardia). If appropriate equipment is available, LV size should be evaluated with planimetry to measure 2-dimensional chamber area or volume at end-systole and end-diastole. Linear measures of end-diastolic and end-systolic diameter can produce spurious results with regional injury models and should be used cautiously. To index hypertrophy, LV wall thickness should be measured at end-diastole, and relative wall thickness should be included to normalize for chamber size. LV systolic function by echocardiography is best assessed by LVEF or fractional area change, although single-dimension fractional shortening is also reasonable with global injury models. The imaging data should be supported by gravimetric data to show chamber hypertrophy and/or elevation of filling pressure (eg, wet lung weight), and ideally by mechanical data demonstrating depressed contractility and lusitropy and elevated filling pressure.

There are shortcomings to all HF animal models that limit their relevance to disease in humans. For example, the manifestations of clinical HF (reduced blood flow and elevated cardiac filling pressure) are often temporarily removed from the onset of pathological remodeling.\textsuperscript{35} This is also the case in most DCM animal models. Therefore, it is possible to have significant remodeling in an animal model without severe clinical signs (eg, asymptomatic LV dysfunction).\textsuperscript{3,32,34} Studies in animals at these early stages would be more accurately classified as an examination of pathological remodeling during early HF. In addition, although the DCM phenotype shares multiple similarities regardless of the inciting etiology, there are differences specific to the underlying etiology that should be considered. For example, tachycardia-induced cardiomyopathy in large animals is reversible to some extent on reversal of the tachycardia.\textsuperscript{72,73} Moreover, myocyte hypertrophy and fibrosis do not feature prominently in this form of cardiomyopathy despite changes in hemodynamics, neurohormonal activation, and chamber structure and function that replicate clinical DCM.\textsuperscript{73–75}

**Animal Models Currently Used for the DCM Phenotype**

A variety of animal models (large and small) have been used to mimic the human DCM phenotype and HF. Several of these models have been the subject of recent reviews.\textsuperscript{76–80}

**Rodent DCM Models**

Rodent models are available for studies of DCM. They are relatively inexpensive (compared with large-animal models),
and manipulation of mouse genetics allows gain or loss of function of specific genes in specific cell types at specific times. These features allow for experimental designs that evaluate specific molecular mechanisms in greater animal numbers with more substantial statistical power. However, there are critical structural, functional, and molecular differences between small and large mammalian hearts, such that promising therapeutic approaches generally require preclinical testing in larger-animal models before human translation.

**Ischemic Injury/Myocardial Infarction**

DCM can be induced in rodents by surgical interruption of coronary arteries to produce myocardial infarction via either permanent coronary ligation or reperfused infarction (ischemia/reperfusion). After an infarction, the DCM phenotype progressively develops. It is essential to recognize that, in these models, the degree of long-term LV remodeling and chamber dilatation is directly proportional to the initial infarct size. Therefore, it is necessary to demonstrate equivalence of infarct size between groups when comparing subsequent remodeling responses in different groups of animals. Cryo injury is often used as an alternative technique to interrupt coronary blood flow because it can give a more reliable area of injury.

**Transgenic Overexpression and Knockout Models**

Animals with constitutive and inducible transgenic overexpression and gene knockout models that exhibit a DCM phenotype are available for study. The penetrance and gene knockout models that exhibit a DCM phenotype are highly specific forms of injury and also can be useful in assessing cardiac responses to stress.

**Toxic Models**

A DCM phenotype has been induced with doxorubicin or isoproterenol. These approaches can produce a dose-dependent dilated phenotype and HF over time after sufficient myocardial injury and cell death. These models are characterized by myocyte apoptosis and oxidant stress. Toxic models of cardiomyopathy are highly specific forms of injury and also can be useful in assessing cardiac responses to stress.

**Other Causes**

Hypertensive, pressure overload, and volume overload rodent models of DCM are also available, and these are discussed in other portions of this statement. The spontaneous hypertensive rat also develops HF, and this model can be useful for defining causes and putative new therapies.

**Large-Animal DCM Models**

Preclinical validation of novel therapeutic approaches usually requires large-animal models because they more closely approximate human cardiac structure and physiology. Furthermore, testing of device therapies are not easily performed in small-animal models. In addition, structural, hemodynamic, and physiological assessments can often be made with much less invasive approaches in large animals. DCM can be induced in large animals by myocardial infarction, coronary microembolization, pacing-induced tachycardia, and toxic injury. These models can be used to define hemodynamic, mechanical, neurohormonal, cellular, and molecular changes during HF and to evaluate the potential efficacy of novel therapeutics.

**Coronary Ligation/Regional Myocardial Infarction**

DCM infarction studies (both reperfused and nonreperfused) have used dogs, pigs, and sheep to evaluate the pathophysiological mechanisms of postinfarction remodeling and DCM development and progression, and the response to therapies. Posterior infarction models (eg, ligation of the posterior descending artery and distal branches of the circumflex artery) have been used to study the role of ischemic MR in postinfarction remodeling. Importantly, dogs have a well-developed collateral circulation in comparison with pigs, which can result in higher variability in infarct size and subsequent remodeling, making the use of canine myocardial infarction models problematic. Porcine and ovine models are characterized by predictable infarction sizes and closely mimic ischemic cardiomyopathy in humans.

**Coronary Microembolization**

Serial left coronary artery microembolization with polystyrene microspheres has been used to induce dilated ischemic cardiomyopathy in dogs and sheep. Acutely microembolized myocardium exhibits contractile dysfunction with a profound perfusion-contraction mismatch, and localized inflammatory responses and TNF expression, as well. Repeated microembolization over a period of 10 weeks induces microinfarcts and progressive LV dilatation and contractile dysfunction (LVEF <35%) resembling human ischemic cardiomyopathy, with neurohormonal activation, natriuretic peptide elaboration, myocyte hypertrophy, MMP upregulation and interstitial fibrosis, and reduced β-AR responsiveness. This model has provided insights into the effects of pharmacological and device-based therapies for HF.

**Pacing-Induced Tachycardia**

Chronic tachycardia-mediated DCM is a recognized clinical condition. In dogs, pigs, and sheep, rapid pacing of either the atrium or the ventricle for at least 3 to 4 weeks produces a progressive, reliable, and reproducible model of DCM and chronic HF, that is at least partially reversible over time on discontinuation of pacing. This disease model closely replicates the mechanical, structural, neurohormonal, and myocyte functional alterations of DCM in humans and has been used to test pharmacological and gene-based therapies. The predictability and reproducibility of the model, and its parallels to the hemodynamic and mechanical phenotype of DCM in humans, render this an attractive model. Limitations include the absence of myocyte hypertrophy and fibrosis at the tissue level and the reversible nature of this myopathy.

**Toxic Models**

Serial administration of intracoronary and intravenous doxorubicin induces toxic DCM in dogs, sheep, and, more recently, in cows. As in rodents, doxorubicin cardiotoxicity is dose dependent and characterized by myocyte injury, myocyte and endothelial cell loss and apoptosis.
felt to be critical to the development of novel therapies for device therapeutics in well-characterized large-animal models is addition, testing pharmacological, gene-based, cell-based, and ischemic and load-dependent DCM are of great relevance. In defining critical causes of DCM in large-animal models of humans are ischemic heart disease and hypertension. Therefore, should also include histopathologic, biochemical, cellular, and the bases of any beneficial effect of a tested therapeutic. Most studies which genes can be modified in these animals is their major strength. The limitations of these models are that they are far removed from the complexity of the adult mammalian heart.

**Recommendations**

DCM animal models should exhibit the structural and mechanical alterations of DCM in humans and also share many of neurohormonal, cellular, and molecular features that were detailed above. The central features of the model should include ventricular dilatation and relative wall thinning with eccentric hypertrophy, depressed contractility and lusitropy, and diminished contractile/lusitropic reserve with stress, all leading to the systemic manifestations of HF (reduced output/flow and elevated filling pressure). Phenotypic assessment should typically include morphological assessment via echocardiography (and/or cardiac magnetic resonance imaging [MRI] if available) and gravimetry. Most studies should use invasive in vivo measurement of cardiac pressures and mechanics to critically evaluate cardiac function, especially if the study seeks to evaluate therapeutics that are thought to improve systemic HF. Isolated myocyte, muscle, or perfused heart preparations can also be used when animals are euthanized to define myocyte contraction status. Most studies should also include histopathologic, biochemical, cellular, and molecular studies to document a human DCM phenotype and the bases of any beneficial effect of a tested therapeutic.

The most common underlying causes for acquired DCM in humans are ischemic heart disease and hypertension. Therefore, defining critical causes of DCM in large-animal models of ischemic and load-dependent DCM are of great relevance. In addition, testing pharmacological, gene-based, cell-based, and device therapeutics in well-characterized large-animal models is felt to be critical to the development of novel therapies for patients experiencing DCM. Primary (idiopathic) DCM is often the result of genetic mutations, many of which are undiscovered. Mechanism discovery for genetically based DCM is ideally performed in transgenic and knockout mice, and initial evaluation of novel molecular mediators or targets in DCM is best facilitated by the generation of genetically modulated mouse models for the molecule of interest. The expression levels and function of the specific target should also be determined in acquired DCM models or human hearts to help establish disease relevance.

**Hypertensive Heart Disease**

**Description of the Clinical Entity**

Hypertensive heart disease (HHD) is a major public health problem that contributes importantly to cardiovascular morbidity and mortality. This is particularly the case in the black population, where LVH is 2- to 3-fold more common than in the general population. The overarching concept in this field is that, with persistent hypertension, or pressure overload, there is a transition from compensated hypertrophy to HF. There are substantial data, both in animal and human studies, that support this concept. The operative principle is that HHD is initially characterized by concentric hypertrophy, typically with a normal EF and normal or decreased end diastolic volume (similar to AS, as described earlier). With progression of the syndrome, there is often an increase in LV end-diastolic and end-systolic volume and decreased EF (Figure 3). This is an ominous sign and is usually associated with signs and symptoms of systolic HF. It is also clear that increases in LV chamber stiffness and/or impaired active relaxation associated with pathological myocyte hypertrophy and matrix remodeling can impair LV filling, raise LV filling pressure, and induce the syndrome of HF without a major decrease in LVEF.

There are still many unknowns regarding how HHD patients transition from a phase of compensated hypertrophy to HF. Large, longitudinal cohort studies in humans with HHD who have undergone sequential imaging are needed if we are to be better informed regarding the relative likelihood of a transition to HF with low EF versus HF with preserved EF among patients with hypertension. It is also possible that elevated blood pressure in humans leads to a dilated HF phenotype without a phase of concentric LVH. The lack of critical information in humans makes it difficult to know the critical features of an appropriate HHD animal model.

Among HHD patients with concentric LVH, some manifest reduced regional systolic function, because midwall microvascular insufficiency, and oxidative stress. Use of this DCM model in sheep and cows can provide an experimental platform for evaluating the effects of mechanical circulatory support devices. Limitations of this model include variability of response to doxorubicin and the degree of LV dysfunction, animal mortality caused by arrhythmias, and the potential for systemic, gastrointestinal, and bone marrow side effects.

**Fly and Fish Models**

This statement focuses on mammalian models of HF. The readers should be aware that fly\(^{137}\) and fish\(^{138}\) models are available. These animal models are particularly well suited for studies exploring the role(s) of specific genes in the development, progression, or prevention of HF. These models are also useful for studies of cardiac regeneration.\(^{139}\) The ease with which genes can be modified in these animals is their major strength. The limitations of these models are that they are far removed from the complexity of the adult mammalian heart.

**Figure 3.** Left ventricular geometric patterns in hypertensive patients. Echocardiography shows that the left ventricle can adapt any 1 of 4 geometric patterns in response to hypertension, reflecting the relative contributions of pressure and volume overloads. LVM indicates left ventricular mass index; RV, right ventricle; LV, left ventricle; LVH, left ventricular hypertrophy; d, left ventricular chamber diameter; e, left ventricular wall thickness. Reprinted from Ganau et al,\(^{2}\) with permission from Elsevier.
fractional shortening can be impaired even with preserved EF. An important unanswered question is whether LVH with regional systolic abnormalities is a critical prelude to the development of overt systolic failure. Long-term follow-up with sequential imaging studies would be necessary to firmly establish this concept in humans so that appropriate animal models could be developed.

Causes and Associated Features of HHD
The patient with HHD typically is older, commonly black, more likely to be female, and more often manifests obesity and type 2 diabetes mellitus. Coronary disease is common in such patients, and there is a 6-fold increase in the prevalence of myocardial infarction. Among hypertensive patients who develop HHD, treatment has frequently been inadequate. Some element of renal insufficiency is not uncommon because of hypertensive nephrosclerosis, and this risk is greater with concomitant diabetes and/or atherosclerosis. Thus, HHD in humans is a complex multifactorial process that often leads to HF, with all of its classical signs and symptoms. Echocardiography can be used to assess the LV morphology and mass, LVEF, the presence or absence of regional contractile abnormalities, and the presence, pattern, and degree of diastolic dysfunction.146,147

Other imaging techniques used to assess the phenotype of HHD in patients include MRI, which is especially useful to demonstrate patchy replacement fibrosis along with most of the findings identified via echocardiography. Cardiac catheterization with angiography is sometimes performed to assess coronary vasculature.

Circulating biomarkers increased in HHD include B-type natriuretic peptide, N-terminal pro-B-type natriuretic peptide, and troponin I or troponin T levels. There is general agreement that elevation of these biomarkers is related to the severity of HF and is associated with a poor diagnosis.

Critical Features of an Animal Model of HHD
Because the spectrum of HHD in humans is varied, complex and multifactorial (Table 1), it is clear that a given animal model of HHD-induced HF can only reproduce selected elements of the phenotype. Indeed, the ability to exploit the relative consistency of animal models to increase our understanding of a variable clinical entity is a major justification for animal experimentation. Moreover, because most patients with hypertension and LVH are at risk to develop HF (stage B), animal models that represent that different stages of HHD can be useful for studying disease progression and for testing novel therapeutics that could improve cardiac structure and function.

Animal models of HHD should have critical characteristics of the disease in humans, including arterial hypertension, an increase in LV mass, and characterist changes in LV geometry. Cardiac performance should initially be maintained, but eventually diastolic and/or systolic dysfunction should be present. These changes may either be demonstrated by use of echocardiography, MRI, or catheter-based techniques as used in humans. Large-animal models with LV structural and functional impairment may develop a human-like condition of HF, including cough, exercise intolerance, and ascites. These features are more difficult to faithfully demonstrate in small animals. Peripheral biomarkers may complement the assessment of animal models of HHD by identifying relevant pathophysiological processes and clarifying the stage and/or severity of disease. Changes in the structure and/or function of myocytes, the interstitium, and the vasculature should also be documented (Figure 4). At the myocyte level, pathological hypertrophy is associated with activation of calcineurin, nuclear factor of activated T-cell signaling.148 The reader should be aware that this statement does not address right ventricular hypertrophy and failure that results from hypoxia or pulmonary hypertension.149

These are important clinical problems, and there are animal models of these conditions.

Current Animal Models
Many different animal models that mimic HHD have been used over the years to gain insight into the complex biology of this clinical problem. These studies have shown that the transition from concentric LVH to HF can be demonstrated in animal models, including the in spontaneously hypertensive rat,150 in aortic banding,151 and in mice with genetic alterations of various molecules.148

A dog model of HHD produced by wrapping 1 kidney in silk and subsequently performing contralateral nephrectomy

Table 1. Clinical Hypertensive Heart Disease Findings: Human Phenotype With Heart Failure

<table>
<thead>
<tr>
<th>Feature</th>
<th>Example</th>
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<tbody>
<tr>
<td>High blood pressure (&gt;140/90 mm Hg; may be normal in end stages)</td>
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<tr>
<td>Breathlessness at rest or with minimal activity</td>
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<tr>
<td>Fatigue</td>
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<tr>
<td>Edema</td>
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<tr>
<td>LVH (concentric hypertrophy with cardiac myocytes demonstrating an</td>
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<tr>
<td>increased diameter) or increased LV mass</td>
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</tr>
<tr>
<td>Impaired active relaxation</td>
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<tr>
<td>Impaired passive filling</td>
<td></td>
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<tr>
<td>Impaired regional midwall systolic function</td>
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<tr>
<td>Impaired EF (late)</td>
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<tr>
<td>Dilated LV internal chamber (late)</td>
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<tr>
<td>Mitral regurgitation</td>
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<tr>
<td>Enlarged left atrium</td>
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<tr>
<td>Atrial fibrillation</td>
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<td>Conduction abnormalities</td>
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<td>Ventricular arrhythmias</td>
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<tr>
<td>Concomitant coronary artery disease</td>
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<tr>
<td>Previous myocardial infarction</td>
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<tr>
<td>Myocardial fibrosis (reactive and replacement)</td>
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<td>Abnormal pressure/volume relationship in the LV with increased LV</td>
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<tr>
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<tr>
<td>Decreased intramyocardial capillary density</td>
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<tr>
<td>Coronary arteriolar thickening</td>
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<tr>
<td>Decreased coronary blood flow reserve</td>
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<tr>
<td>Increase biomarkers such as NT-proBNP, BNP, and troponin</td>
<td></td>
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<tr>
<td>Obesity, type 2 diabetes mellitus insulin resistance</td>
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LVH indicates left ventricular hypertrophy; LV, left ventricular; EF, ejection fraction; BNP, B-type natriuretic peptide; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.
Table 2. Critical Features of an HHD Animal Model

<table>
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<th>Feature</th>
<th>Description</th>
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HHD indicates hypertensive heart disease; LVH, left ventricular hypertrophy; LV, left ventricular; EF, ejection fraction; BNP, B-type natriuretic peptide; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

has been used previously. Recently, a variant of this model has been used and is one well-established approach to producing a large-animal model of HHD.

Recommendations

There are still considerable gaps in our understanding of HHD, and many of these are best addressed in small- or large-animal models of HHD. For example, 1 hypothesis is that LVH is compensatory and prevents the development of dilated HF. However, some studies in animal models suggest that prevention of the LVH normally induced by pressure overload does not promote dilated cardiac failure and may prevent HHD. This is an area that can be studied further in small- and large-animal models of HHD to develop and test novel therapeutic targets. The natural history of HHD in animal models should be defined longitudinally to determine which features of human HHD are present. These studies should define the proportion of HHD animals that develop a reduction in LVEF and proceed from HHD to dilated HF.

Frequently, HHD in humans is associated with concomitant coronary artery disease with myocardial infarction, diabetes mellitus, metabolic syndrome, conduction system–induced ventricular dyssynchrony, or impaired filling of the LV because of reduced chamber distensibility. Animal studies with more complex etiologies of HF could be developed to address these issues and to test novel therapeutics as they are developed.

Restrictive Cardiomyopathy

Description of Clinical Entity

Restrictive cardiomyopathies are predominantly defined by a physiological dynamic in which relatively small or normal increases in ventricular filling volumes are associated with exaggerated increases in diastolic pressures. Typically, this restrictive ventricular filling pattern is associated with a normal ventricular EF. Anatomically, the LV and right ventricle chamber sizes are usually normal, and wall thickness is normal or mildly increased. Biventricular dilatation is usually present because of chronically increased ventricular diastolic pressures in both ventricles. Indeed, massive biventricular enlargement combined with normal or reduced ventricular chamber size is a classic morphological pattern among patients with restrictive cardiomyopathies. Clinical presentations of patients with restrictive cardiomyopathy are characterized by dyspnea resulting from elevated diastolic pressures, prominent signs of fluid retention, and often fatigue and weakness reflective of impaired cardiac output reserve, but no evidence of cardiomegaly on chest radiography.

This clinical presentation of acquired restrictive cardiomyopathy can be similar to that of constrictive pericarditis, although the underlying origin of the syndrome is very distinct.

Causes and Associated Features

Etiologies of restrictive cardiomyopathy include sarcoidosis, eosinophilic cardiomyopathy, endomyocardial fibrosis, scleroderma, radiation-induced fibrosis, familial restrictive cardiomyopathies, amyloidosis, hemochromatosis, and idio-
pathic restrictive cardiomyopathy. A common feature of many acquired restrictive cardiomyopathy etiologies is a predominant remodeling of the myocardial extracellular matrix via either pathological protein deposition or an aggressive fibrotic process resulting from diffuse myocyte cell death. Although potentially present, defects in cardiac myocyte physiology per se have not been described among patients with these acquired restrictive cardiomyopathies. However, for inherited restrictive cardiomyopathies, several recent studies demonstrate that specific sarcomeric protein mutations are associated with defects in myocardial function and increased myofilament calcium sensitivity. Mutations involving cardiac troponin I (cTnI), cardiac troponin T, desmin, and α-β-crystallin have been most often associated with a restrictive cardiomyopathy phenotype, although alternative mutations of these proteins can also produce a hypertrophic cardiomyopathy phenotype. The histological abnormalities observed in restrictive cardiomyopathies vary with, and are often diagnostic of, the underlying etiology. For example, demonstration of amyloid or iron deposition within the myocardium is diagnostic of amyloidosis and hemochromatosis, respectively.

The onset of restrictive cardiomyopathy during childhood in the absence of extracardiac abnormalities strongly suggests a primary genetic etiology. However, some familial restrictive cardiomyopathies are not apparent until adulthood. Atrial fibrillation is seen with many etiologies of restrictive cardiomyopathy. Ventricular arrhythmias are particularly prevalent among patients with sarcoidosis and some of the mutations associated with familial restrictive cardiomyopathy. Cardiac conduction defects often accompany amyloidosis. Although patients with restrictive cardiomyopathy may present with acute HF after arrhythmias or volume overload, most of the etiologies involved exert their detrimental effects on myocardial performance over the course of many months or years. With the exception of some cases of iron overload cardiomyopathy following iron chelation therapy and the control of some cases of cardiac amyloidosis with stem cell transplantation and/or chemotherapy, the great majority of restrictive cardiomyopathies are progressive and associated with a poor prognosis. Survival is <50% at 5 years after diagnosis.

### Critical Features of an Animal Model of Restrictive Cardiomyopathy

A clinically relevant animal model of restrictive cardiomyopathy must have a documented increase in ventricular chamber stiffness as manifested by an exaggerated increase in LV diastolic pressure in response to a volume challenge. Increased myocardial passive stiffness during in vitro testing should also be documented. Atrial enlargement is another critical feature that should be present to document that increases in ventricular filling pressures have been sustained over time.

Because increased chamber stiffness can be seen in severe cases of hypertrophic or DCM, the absence of marked myocardial hypertrophy (increased voltage on ECG, increased wall thickness without substantial myocyte hypertrophy), and the absence of LV dilation are additional essential distinguishing criteria for animal models of restrictive cardiomyopathy.

For an animal model of restrictive cardiomyopathy to be considered a heart failure model, evidence of progressive impairment of cardiac functional reserve, increased mortality attributable to cardiac causes, and extracardiac features of the HF syndrome such as fluid retention (increased lung weight to body weight, edema, ascites) and neurohormonal activation such as increased natriuretic peptides should be documented. If possible, there should be studies that show that the characteristic restrictive increase in LV chamber stiffness is associated with reduced exercise tolerance or a pathological response (reduced natriuresis and/or pulmonary edema) following a volume challenge. Documenting all of these features of a restrictive cardiomyopathy in an animal model would allow novel mechanistic features under investigation to be related to characteristic phenotypic features of the disease in humans or for therapeutics to be related to these same features.

### Restrictive Cardiomyopathy: Current Animal Models

Because diverse etiologies can produce restrictive cardiomyopathies, the potential animal models associated with this phenotype are likewise diverse. As illustrated in Table 3, the majority of the animal models of restrictive cardiomyopathy described to date are rodent models with a relatively few large-animal models reported. It is convenient to segregate these models into those that are related to acquired restrictive cardiomyopathies and those modeling familial cardiomyopathies. In theory, the clinical relevance of an animal model of an acquired restrictive cardiomyopathy is enhanced when it replicates the relationship between an exposure and a tissue abnormality (eg, iron overload/iron deposition or radiation exposure/radiation-induced vascular and myocardial injury) that is observed in clinical settings. On the other hand, the clinical relevance of an animal model of a familial restrictive cardiomyopathy is likely greatest when the mutation produced in a genetically modified mouse strain is identical or

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**Table 3. Animal Models of RCM**

<table>
<thead>
<tr>
<th>Animal Models of RCM</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodent models of acquired RCM</td>
<td>181–183</td>
</tr>
<tr>
<td>Iron overload</td>
<td>184–186</td>
</tr>
<tr>
<td>Radiation fibrosis</td>
<td>187</td>
</tr>
<tr>
<td>Eosinophilic myocarditis</td>
<td>188–193</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>194–196</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>195–197</td>
</tr>
<tr>
<td>Rodent models of hereditary RCM</td>
<td>198, 199</td>
</tr>
<tr>
<td>Hereditary hemochromatosis</td>
<td>200–201</td>
</tr>
<tr>
<td>Sarcomeric protein mutations</td>
<td>202, 203</td>
</tr>
<tr>
<td>Large-animal models</td>
<td>204</td>
</tr>
<tr>
<td>Spontaneously occurring RCM in cats</td>
<td>205</td>
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<tr>
<td>Amyloidosis in aged vervet monkeys</td>
<td>206</td>
</tr>
<tr>
<td>Bovine systemic AA amyloidosis</td>
<td></td>
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<tr>
<td>Amyloidosis in Abyssinian cats</td>
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</table>

RCM indicates restrictive cardiomyopathy.
analogous to a mutation known to be associated with familial restrictive cardiomyopathy in humans.

In rodent models of acquired restrictive cardiomyopathy, investigators have used simple analogues of clinically detrimental exposures to produce acquired hemochromatosis and radiation-induced myocardial disease or immune sensitization and adoptive cell transfer to produce eosinophilic myocarditis. Although scleroderma (also known as systemic sclerosis) is an etiology of acquired restrictive cardiomyopathy, numerous studies used the tight skin (Tsk) mouse as a model of scleroderma before and after the recognition that fibrillin-1 overexpression is responsible for the excessive fibrosis associated with this strain. In a like manner, mice overexpressing amyloidogenic proteins, such as transthyretin, have been used to mimic systemic amyloidosis. Only a few studies focusing on exogenous iron overload and radiation-induced myocardial injury have demonstrated that the animal model demonstrates critical features of restrictive cardiomyopathies in humans.

Rodent models of familial restrictive cardiomyopathy have generally focused on mimicking the clinical entities of hereditary hemochromatosis or restrictive cardiomyopathies associated with sarcomeric protein mutations. For hereditary hemochromatosis, murine models with homozygous knockout of hemoujuvelin (JUV) have produced the most consistent and marked increases in myocardial iron deposition, whereas studies using HFE gene knockout mice have required administration of a high-iron diet to induce myocardial iron deposition. These models replicate genotypes observed in humans with familial hemochromatosis, but, as yet, there is little evidence for critical physiological features of restrictive cardiomyopathy leading to HF.

To date, 2 different cTnI mutations have produced mouse models to mimic familial restrictive cardiomyopathy in humans. In 1 model, transgenic mice carrying the R193H mutation (analogous to the R192H mutation associated with restrictive cardiomyopathy in humans), developed atrial dilatation with reduced LV chamber size and increased chamber stiffness in the absence of increased wall thickness. In comparison with nontransgenic littermates, mice with the R193H cTnI mutation demonstrate a reduced resting cardiac output, reduced contractile reserve in response to dobutamine, and a higher mortality by 12 months of age. Studies in the cTnI R145W mouse have shown that the same cTnI locus can be associated with either a hypertrophic or a restrictive cardiomyopathy phenotype (in both humans or mice) depending on which base pair is substituted at the locus. In this case, isolated cardiac muscles fibers from the mutation associated with the restrictive cardiomyopathy phenotype exhibited increased Ca\(^{2+}\) sensitivity, a 40% increase in peak force and prolonged force, and [Ca\(^{2+}\)]\(_{t}\) transients that would tend to impair ventricular filling. These studies validate that the animal model has the salient features of restrictive cardiomyopathy in humans.

As highlighted in Table 3, the large animal models of restrictive cardiomyopathy consist mainly of naturally occurring states that have been reported by investigators. For the most part, the characterization of these models has been limited to pathological and histological analysis without characterization of myocardial/chamber stiffness. Although models using purposeful exposure of large animals to iron overload or cardiac irradiation could be developed and characterized, we cannot find evidence of such efforts to date.

**Recommendations**

There are small- and large-animal models for study of the causes and consequences or for the treatment of restrictive cardiomyopathy. Functional demonstration of increased myocardial chamber stiffness leading to the syndrome of HF caused by a restrictive cardiomyopathy should be a central feature of future studies with these animal models.

**Methodological Recommendations**

HF animal models must be carefully characterized to ensure that they have the critical features that have been described above. The level of characterization will ultimately be determined by the study design and the available equipment and resources. The approaches that have been used routinely to reliably characterize large- and small-animal HF models are outlined below.

**Evaluation of Large-Animal Models**

Large-animal models continue to be a mainstay for drug, cell, and gene therapy development, and for device development and surgical procedure testing, as well. There has been nearly half a century of technological development for assessing cardiovascular function in large animals. Importantly, essentially anything that can be used in humans can be applied to large-animal models and should be considered. Furthermore, in large-animal models it is possible to obtain data in the conscious rather anesthetized state, so the hemodynamic characterization is more physiological. This also allows measurements to be made over time in the same animal.

The methods that are recommended to assess large-animal cardiovascular function include (1) implantable sensors that can be used chronically in conscious animals, (2) catheters and other sensors that are used acutely under anesthesia or sedation, (3) noninvasive imaging methodologies that may be used in conscious or sedated animals depending on the method. The types of measurements made in any given study will depend on the goals of the analysis, and measurements can be simple or quite complex. Importantly, the principals and approaches are all well established.

**Implantable Sensors**

These techniques require a surgical intervention for insertion of instrumentation before the induction of the HF stressor. Typically, one combines a series of sonomicrometers implanted into the LV (or right ventricle) to assess dimensions and thereby calculate chamber volume and wall thickness. Micromanometers can be inserted into arterial, venous, and ventricular cavities to measure pressure, ultrasound probes can be used to record central and regional blood flow, fluid-filled catheters can be inserted for calibration, pacing wires can be used to control heart rate, and inferior vena cava cuff occluders can be used to modulate preload volume. With this classic instrumentation, cardiac performance can be assessed by using pressure-volume relations, which facilitates the dissection of primary systolic and...
diastolic properties of the heart from vascular loading influences. Cardiac power, dP/dt, systemic resistance, aortic input impedance, and essentially any mechanical parameter that can be derived from pressures, volumes, and flows are available. Recording can be made by connecting a single electric plug to a skin-button where all internal sensor wires merge, or by radio telemetry. These approaches allow cardiac structural and functional changes to be measured in conscious animals during the progression of induced disease and after a therapeutic intervention.

**Acute Catheterization**

Studies can be performed by use of catheters much as one would use catheters in a cardiac catheterization laboratory. Sedation and/or full anesthesia is generally required, and, depending on the location, the catheters must be positioned; some imaging tool, such as fluoroscopy, is needed. The most common methods involve placement of balloon-tipped catheters into the pulmonary artery, often combined with a simple imaging approach, such as contrast ventriculography or echocardiography, and micromanometer catheters placed in arterial or ventricular cavities. These methods provide cardiac output, ventricular systolic and diastolic pressures, chamber end-systolic and end-diastolic volume, and EF, but can also be used to derive more complex mechanical parameters. For example, single-beat estimation methods have been reported to assess end-systolic elastance, a measure of maximal chamber stiffening that is otherwise derived from more complex pressure-volume relations. The gold standard catheterization approach for comprehensive analysis of heart and vascular function is again based on pressure-volume relations and arterial/flow relations. Properly calibrated multiphasic electrode conductance (or impedance) catheters developed in the 1980s provide pressure-volume signals simultaneously without the need for imaging and post hoc image processing. These techniques are very valuable for critical evaluation of cardiac status in patients and animal models of all sizes.

**Noninvasive Imaging Modalities**

Nearly all of the imaging methods that can be used in small-animal models are easier to use in larger animals. The exceptions are the molecular imaging methods involving visible or infrared light-emitting reagents that can be visualized in small animals. These methods have been developed to measure contractility by using the end-systolic pressure volume relation in intact mice. Advanced MRI techniques and speckle-tracking strain-related imaging (with 3-dimensional reconstruction) with ultrasound echocardiography have been shown to improve early predictions of outcomes in humans. These new approaches may more accurately measure ventricular volumes and diastolic function in both small- and large-animal models. If MRI is not available, then 3-dimensional transdiaphragmatic echocardiography or 2-dimensional transdiaphragmatic echocardiography should be considered (to alleviate problems with finding useable echocardiography windows).

**Electrocardiograms**

ECGs should be measured in both small- and large-animal models of HF. ECGs are measured routinely in patients to give insight into rate and rhythm disturbances and blood flow abnormalities. This simple technique should be used routinely at the time of euthanasia in studies of all HF animal models.

**Small-Animal Models**

**In Vivo Cardiac Function**

The noninvasive technique of transthoracic echocardiography can be used to evaluate cardiac function in anesthetized or conscious mice. Transthoracic 2-dimensional guided M-mode echocardiography can be performed by using a variety of commercially available echocardiograph machines. LV end-diastolic and end-systolic dimensions, heart rate, velocity of circumferential shortening, and percentage of fractional shortening should be determined. Serial measurements should be performed to evaluate structural and functional progression of HF or to evaluate the efficacy of therapeutics.

**In Vivo Measurements of Intrinsic Contractile Function**

Since loading conditions can affect isovolumic phase indices of contractility, such as LV dP/dtmax, and ejection phase indices, such as fractional shortening and EF, techniques have been developed to measure contractility by using the end-systolic pressure volume relation in intact mice. Established methods most commonly use miniaturized conductance micromanometry. Cardiac catheterization should be performed by using a small conductance catheter inserted retrograde through the right carotid artery into the LV or through an apical stab. Loading conditions should be varied by either inferior vein occlusion or with transient TAC. Modification of these methods can be used to measure myocardial stress-strain relations in normal and “diseased” mice in vivo.

**In Vivo β-AR Responsiveness**

Isovolumic phase indices of contractility (such as LV dP/dt max) have limited sensitivity when comparing groups of different animals. However, LV dP/dtmax provides useful information on relative changes in contractile behavior when conditions are controlled in the same animal. A change in maximal and minimal first derivative of LV pressure (LV dP/dt max, min) in response to catecholamine stimulation is an excellent and sensitive measure of in vivo β-AR func-
tion. The methodology for cardiac catheterization in the mouse is now considered routine and has been performed in many laboratories around the world. A high-fidelity 1.0F or 1.4F micromanometer catheter is inserted into a carotid artery and advanced into the LV. Following bilateral vagotomy, continuous high-fidelity LV pressure can be recorded at baseline and following bolus doses of a catecholamine such as isoproterenol or dobutamine. Parameters measured are heart rate, aortic pressure, LV systolic and diastolic pressure, and the LV dP/dtmax, min. These approaches should be considered to evaluate basal contractile defects and alteration in contractility reserve.

Disclosures

Writing Group Disclosures

<table>
<thead>
<tr>
<th>Writing Group Member</th>
<th>Employment</th>
<th>Research Grant</th>
<th>Other Research Support</th>
<th>Speakers’ Bureau/Honoraria</th>
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<th>Ownership Interest</th>
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<td>Howard A. Rockman</td>
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<td>Founder and Chief Scientific Officer of CardioCreate Inc.†</td>
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*Modest.
†Significant.

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<tr>
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<th>Other Research Support</th>
<th>Speakers’ Bureau/Honoraria</th>
<th>Expert Witness</th>
<th>Ownership Interest</th>
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<td>Johnson and Johnson†</td>
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</table>

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*Modest.
†Significant.
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Animal Models of Heart Failure: A Scientific Statement From the American Heart Association

Steven R. Houser, Kenneth B. Margulies, Anne M. Murphy, Francis G. Spinale, Gary S. Francis, Sumanth D. Prabhu, Howard A. Rockman, David A. Kass, Jeffery D. Molkentin, Mark A. Sussman and Walter J. Koch

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In the article by Houser et al, “Animal Models of Heart Failure,” which published ahead of print on May 17, 2012, and appeared in the June 22, 2012, issue of the journal (Circ Res. 2012;111:131–150.) several corrections were needed:

1. On page 131, the author listing paragraph read, “. . .Walter Koch, PhD, FAHA; . . .” It has been changed to read, “. . .Walter J. Koch, PhD, FAHA; . . .”

2. On page 131, in the footnotes, third paragraph, the citation read, “Houser SR, . . . Koch W; on behalf of . . . .” It has been changed to read, “Houser SR, . . . Koch WJ; on behalf of . . . .”

3. On page 144, in the Disclosure Table, under the “Writing Group Member” column, the fifth row read, “Walter Koch.” It has been changed to read, “Walter J. Koch.”

These corrections have been made to the print version and to the current online version of the article, which is available at http://circres.ahajournals.org/content/111/1/131.full.