The Control of Diastolic Calcium in the Heart
Basic Mechanisms and Functional Implications

David A. Eisner, Jessica L. Caldwell, Andrew W. Trafford, David C. Hutchings

**ABSTRACT:** Normal cardiac function requires that intracellular Ca$^{2+}$ concentration be reduced to low levels in diastole so that the ventricle can relax and refill with blood. Heart failure is often associated with impaired cardiac relaxation. Little, however, is known about how diastolic intracellular Ca$^{2+}$ concentration is regulated. This article first discusses the reasons for this ignorance before reviewing the basic mechanisms that control diastolic intracellular Ca$^{2+}$ concentration. It then considers how the control of systolic and diastolic intracellular Ca$^{2+}$ concentration is intimately connected. Finally, it discusses the changes that occur in heart failure and how these may result in heart failure with preserved versus reduced ejection fraction.

**Key Words:** calcium ♦ diastole ♦ heart failure ♦ myofibrils ♦ stroke volume

---

**REVIEW**

**The Control of Diastolic Calcium in the Heart**

**Basic Mechanisms and Functional Implications**

David A. Eisner, Jessica L. Caldwell, Andrew W. Trafford, David C. Hutchings

**ABSTRACT:** Normal cardiac function requires that intracellular Ca$^{2+}$ concentration be reduced to low levels in diastole so that the ventricle can relax and refill with blood. Heart failure is often associated with impaired cardiac relaxation. Little, however, is known about how diastolic intracellular Ca$^{2+}$ concentration is regulated. This article first discusses the reasons for this ignorance before reviewing the basic mechanisms that control diastolic intracellular Ca$^{2+}$ concentration. It then considers how the control of systolic and diastolic intracellular Ca$^{2+}$ concentration is intimately connected. Finally, it discusses the changes that occur in heart failure and how these may result in heart failure with preserved versus reduced ejection fraction.

**Key Words:** calcium ♦ diastole ♦ heart failure ♦ myofibrils ♦ stroke volume

---

In keeping with the above quotation from the collection of poems for children by A.A. Milne, the focus of this article is not on the extensively studied mechanisms that deliver calcium ions to the myofilaments and thereby produce systole. Rather, we review the much less well understood removal of Ca$^{2+}$. Specifically, we will consider how diastolic intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) is controlled and how it changes in disease.

**WHY IS IT IMPORTANT TO CONTROL DIASTOLIC [Ca$^{2+}$]?,?**

**Mechanical Relaxation**

An upper limit for diastolic [Ca$^{2+}$] results from the need for the myofilaments to be deactivated to allow ventricular filling. There may, however, be reasons for ensuring that diastolic [Ca$^{2+}$], is not too low as, the lower it is, to reach a given systolic level, more Ca$^{2+}$ must be added to and removed from the cytoplasm on each beat. This will increase energy expenditure, and since Ca$^{2+}$ cycling accounts for about 30% of the energy consumption of the myocardium, this may be a significant factor in requiring that diastolic [Ca$^{2+}$] is not too low.

**Diastolic Influences Systolic [Ca$^{2+}$], and Force**

There are 2 factors. (1) The lower the diastolic [Ca$^{2+}$], the more Ca$^{2+}$ must be added to produce a given increase in [Ca$^{2+}$]. This is because, at low [Ca$^{2+}$], the cytoplasmic Ca$^{2+}$ buffers become less saturated and their ability to absorb Ca$^{2+}$ increases. Conversely, as [Ca$^{2+}$] increases, buffering power will decrease so a given increase in [Ca$^{2+}$] will require a smaller increase in total Ca$^{2+}$ (for review, see the article by Smith and Eisner). In other words, by altering the level of saturation of buffers, diastolic [Ca$^{2+}$] determines the amplitude of the systolic transient produced by a given rise of total Ca$^{2+}$ and therefore alterations of diastolic [Ca$^{2+}$] change the inotropic response. (2) A further consideration is that force depends steeply on [Ca$^{2+}$], so that, starting from an elevated diastolic [Ca$^{2+}$], a smaller increase in [Ca$^{2+}$] will be required to produce the same change of force compared with at a normal diastolic [Ca$^{2+}$]. Therefore, an increase...
Nonstandard Abbreviations and Acronyms

| Abbreviation | Description |
|--------------|-------------|
| [Ca\(^{2+}\)] \(_i\) | Intracellular Ca\(^{2+}\) concentration |
| [Na\(^+\)] \(_i\) | Intracellular Na\(^+\) concentration |
| CaMKII | Ca\(^{2+}\)/calmodulin-dependent protein kinase II |
| Gd\(^{3+}\) | Gadolinium |
| HFpEF | Heart failure with preserved ejection fraction |
| HFpEF | Heart failure with reduced ejection fraction |
| LV | Left ventricle |
| MCU | Mitochondrial calcium uniporter |
| NCX | Sodium-calcium exchange |
| PKG | Protein kinase G |
| PLN | Phospholamban |
| PMCA | Plasma membrane Ca-ATPase |
| RyR | Ryanodine receptor |
| SERCA | Sarcoplasmic reticulum Ca-ATPase |
| SR | Sarcoplasmic reticulum |
| STIM1 | Stromal interaction molecule 1 |
| TRP | Transient receptor potential |

in diastolic [Ca\(^{2+}\)]\(_i\), will increase the level of developed force produced by a given systolic rise of [Ca\(^{2+}\)]\(_i\).

BASIC MECHANISMS UNDERLYING THE Ca TRANSIENT

The pathways that underlie cardiac calcium cycling are well understood\(^{5,6}\) (Figure 1); the individual mechanisms and their roles in the control of diastolic [Ca\(^{2+}\)]\(_i\), are described in more detail in subsequent sections. Briefly, Ca\(^{2+}\) enters via the L-type Ca channel, and there may also be entry on reverse sodium-calcium exchange (NCX) at the start of the action potential. This Ca\(^{2+}\) entry triggers the release of a larger amount of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) through the ryanodine receptor (RyR)—a process known as calcium-induced calcium release. Ca\(^{2+}\) is then returned to the SR by the SR Ca\(^{2+}\)-ATPase (SERCA), regulated by the accessory protein PLN (phospholamban). At the surface membrane, Ca\(^{2+}\) is removed from the cell by a combination of NCX and PMCA (plasma membrane Ca-ATPase). Finally, the mitochondria can uptake Ca\(^{2+}\) via the MCU (mitochondrial calcium uniporter). The amplitude of the systolic rise of [Ca\(^{2+}\)]\(_i\) is increased by increasing the size of the L-type Ca current\(^{2,9}\) or the amount of Ca stored in the SR.\(^{9,10}\) The latter is determined by the balance of cellular Ca\(^{2+}\) fluxes. For example, increasing SERCA activity or decreasing Ca efflux on NCX will increase SR Ca\(^{2+}\) content. The decay of the Ca transient is largely due to SERCA-mediated reuptake into the SR with, particularly in larger species, significant contributions from NCX.\(^4\) The rate of this decay would be expected to affect end-diastolic [Ca\(^{2+}\)]\(_i\), since, all other things being equal, a faster decay will mean that [Ca\(^{2+}\)]\(_i\) is reduced to a lower level by the time of the next beat, resulting in a lower end-diastolic [Ca\(^{2+}\)]\(_i\).

WHY IS SO LITTLE KNOWN ABOUT DIASTOLIC [Ca\(^{2+}\)]\(_i\)?

There are several reasons for the paucity of data concerning diastolic [Ca\(^{2+}\)]\(_i\). (1) Problems of indicator calibration make it much easier to measure changes than absolute levels of [Ca\(^{2+}\)]\(_i\). This is a particular issue when comparing measurements between cells or animals. (2) When nonratiometric, Ca\(^{2+}\)-sensitive, fluorescent indicators are used, the records are often normalized to the diastolic or resting fluorescence,\(^1\) making it difficult to measure diastolic [Ca\(^{2+}\)]\(_i\). (3) In experiments using the whole cell version of the patch clamp, diffusion of Ca\(^{2+}\) and Ca\(^{2+}\) buffers into or out of the pipette may contribute to regulation of [Ca\(^{2+}\)]\(_i\). Indeed, one of the uses of the whole-cell technique is to control the cytoplasmic ionic concentrations. (4) The major issue may be that, particularly in smaller animals, most experimental work studying Ca\(^{2+}\) cycling in cardiac tissues has used rates of stimulation considerably below normal heart rates. While the fact that ion currents and [Ca\(^{2+}\)]\(_i\) have reached steady state values helps dissect the fluxes responsible for the systolic Ca transient, it establishes an artificial situation. As discussed below, end-diastolic [Ca\(^{2+}\)]\(_i\) represents a balance between many Ca\(^{2+}\)-handling mechanisms. In contrast, in a quiescent myocyte, the resting level of [Ca\(^{2+}\)]\(_i\), is determined entirely by the fluxes of Ca\(^{2+}\) across the sarcolemma\(^{12,13}\) because, in the steady state, there can be no net flux into or out of organelles. Such a net flux would result in a continuous change of organelle Ca\(^{2+}\) content—a situation incompatible with a steady state. At low rates of stimulation, [Ca\(^{2+}\)]\(_i\) will be identical to the resting level seen in the unstimulated case. These frequency-dependent effects are illustrated by making the RyR leaky with caffeine (Figure 2A and 2B). This has no effect on diastolic [Ca\(^{2+}\)]\(_i\) at a stimulation rate of 0.5 Hz but a marked one at 3 Hz.\(^14\) Thus, it is important not to confuse diastolic and resting [Ca\(^{2+}\)]\(_i\). Finally, as discussed below, physiological changes of heart rate result from those of autonomic tone—a factor that is not examined in studies that simply alter pacing rate.

INTERACTION OF CONTROL OF DIASTOLIC AND SYSTOLIC [Ca\(^{2+}\)]\(_i\)

It is tempting to think of the control of diastolic and systolic [Ca\(^{2+}\)]\(_i\) as being separate. From this viewpoint, diastolic [Ca\(^{2+}\)]\(_i\) is controlled at a certain level, and the mechanisms discussed above determine the magnitude
of the systolic rise. We think that this is incorrect; the regulation of diastolic and systolic [Ca\textsuperscript{2+}] is inextricably linked\textsuperscript{,14,15} This has been demonstrated recently by investigating the effects of interfering with SR function on diastolic and systolic [Ca\textsuperscript{2+}].\textsuperscript{16} Consistent with previous data,\textsuperscript{16} making the RyR leaky with caffeine decreased the amplitude of the systolic Ca\textsuperscript{2+} transient by decreasing SR Ca\textsuperscript{2+} content. This was accompanied by an increase in diastolic [Ca\textsuperscript{2+}] such that the average level of [Ca\textsuperscript{2+}] over the cycle was unaffected (Figure 2A).\textsuperscript{14} Similar results were found when SERCA activity was decreased with thapsigargin and were accounted for by considerations of cellular Ca\textsuperscript{2+} flux balance. In the steady state, the Ca\textsuperscript{2+} influx over the cardiac cycle must equal efflux. Interfering with SR function will have no direct effect on influx, and so efflux must also be unaltered. Since Ca\textsuperscript{2+} influx on NCX is proportional to [Ca\textsuperscript{2+}],\textsuperscript{17} constant efflux requires that average [Ca\textsuperscript{2+}], be unaffected, explaining why the decrease of systolic is accompanied by an increase in diastolic [Ca\textsuperscript{2+}]. (Figure 2C). One caveat is required here; interfering with SR function and thereby decreasing the amplitude of the systolic Ca transient can decrease the degree of Ca-dependent inactivation of the L-type Ca current and thereby increase Ca\textsuperscript{2+} influx.\textsuperscript{18,19} In this case, average [Ca\textsuperscript{2+}] would be elevated, potentially elevating diastolic [Ca\textsuperscript{2+}] this does not appear to be an issue in experiments where the RyR was made leaky with caffeine as the L-type Ca\textsuperscript{2+} influx was unaffected.\textsuperscript{14}

**Importance of Average [Ca\textsuperscript{2+}]**

The average [Ca\textsuperscript{2+}] is determined by Ca\textsuperscript{2+} entry and efflux across the surface membrane. An increase in rate will increase Ca\textsuperscript{2+} influx per unit time and thence the average [Ca\textsuperscript{2+}].\textsuperscript{14} Similar effects would be expected for an increase in the amplitude of the L-type Ca current. Conversely, a decrease in the ability of NCX to pump Ca\textsuperscript{2+} out of the cell will increase average [Ca\textsuperscript{2+}] to a level sufficient to maintain Ca\textsuperscript{2+} efflux. This may arise due to either decreased expression of NCX or an increase in the intracellular Na\textsuperscript{+} concentration ([Na\textsuperscript{+}]) and, therefore, a decrease in the energy to pump Ca\textsuperscript{2+} out of the cell (Figure 3). There is an infinite number of combinations of systolic and diastolic [Ca\textsuperscript{2+}], that can establish a given average [Ca\textsuperscript{2+}]. The properties of the SR will be an important factor in determining which occurs. Compromising SR function, by decreasing SERCA activity or increasing Ca\textsuperscript{2+} (leak) efflux through the RyR, will increase diastolic and decrease systolic [Ca\textsuperscript{2+}]. For example, in the presence of a normal SR, \(\beta\)-adrenergic stimulation increases systolic but has no effect on diastolic [Ca\textsuperscript{2+}]. In contrast, when the RyR is leaky, \(\beta\)-stimulation increases diastolic [Ca\textsuperscript{2+}].\textsuperscript{14}
Similar considerations also apply to conditions of calcium overload where waves of calcium release from the SR occur. Much attention has been directed to the detrimental effects of these increases in \([\text{Ca}^{2+}]_i\), which activate NCX\(^{21}\) and thereby produce arrhythmogenic delayed afterdepolarizations.\(^{22}\) However, the resulting Ca\(^{2+}\) efflux will help maintain Ca\(^{2+}\) flux balance and thereby keep diastolic \([\text{Ca}^{2+}]_i\), lower than would otherwise be the case. Under these conditions, addition of caffeine to empty the SR and thereby remove waves and their associated efflux results in a steady level of \([\text{Ca}^{2+}]_i\), which is greater than the minimum seen in the presence of Ca\(^{2+}\) waves.\(^{23}\)

These consequences of flux balance are a generalization of those previously described for changes of systolic \([\text{Ca}^{2+}]_i\), alone.\(^{24–26}\) That work showed that potentiation of RyR opening had no effect on systolic \([\text{Ca}^{2+}]_i\),

---

**Figure 2.** The importance of average intracellular Ca\(^{2+}\) concentration (\([\text{Ca}^{2+}]_i\)) in the control of systolic (syst) and diastolic \([\text{Ca}^{2+}]_i\).

\(A\), The effects of application of caffeine (caff) and stimulation rate on \([\text{Ca}^{2+}]_i\). A rat ventricular myocyte was stimulated at the frequencies shown above and caff (1 mmol/L) applied for the periods denoted by the red bars. The gray trace is the original data and the black denotes average \([\text{Ca}^{2+}]_i\).\(^{14}\) \(B\), Specimen, averaged traces of \([\text{Ca}^{2+}]_i\), from the frequencies shown above. For each frequency, the control (black) and caff (red) traces are superimposed. Data reproduced from Sankaranarayanan et al. \(^{14}\) \(C\), Illustration of flux balance in control and with depressed sarcoplasmic reticulum (SR) function. The total Ca\(^{2+}\) efflux via sodium-calcium exchange (NCX) above control diastolic levels is represented by the area under the \([\text{Ca}^{2+}]_i\) trace. In the depressed SR case, this is separated into 2 components: (1) activated by the syst Ca\(^{2+}\) transient and (2) activated by increased diastolic \([\text{Ca}^{2+}]_i\). Average \([\text{Ca}^{2+}]_i\) is identical with normal and depressed SR (\(A\)), and, therefore, Ca\(^{2+}\) efflux is unchanged and equal to influx.
in the steady state. In those earlier experiments, the decrease of SR Ca content was exactly compensated for by an increase in fractional release from the SR so the amplitude of the Ca transient and the accompanying Ca\(^{2+}\) efflux were unaltered. Ca\(^{2+}\) flux balance could, therefore, be maintained at constant diastolic [Ca\(^{2+}\)]. In the more recent work, the degree of potentiation of the RyR was greater (higher concentrations of caffeine used), and, therefore, the SR Ca content fell to such a low level that even if it is all released, the systolic Ca transient is smaller than control. The consequent decrease of systolic Ca\(^{2+}\) efflux results in systolic efflux being less than influx, thereby loading the cell with Ca\(^{2+}\) and increasing diastolic [Ca\(^{2+}\)]. The increases of diastolic and systolic [Ca\(^{2+}\)] will increase Ca\(^{2+}\) efflux until efflux again equals influx.

In the context of the above general considerations, we will review important aspects of the underlying Ca\(^{2+}\) fluxes before discussing how their integration leads to control of diastolic and systolic [Ca\(^{2+}\)].

**FLUXES REGULATING DIASTOLIC [Ca\(^{2+}\)]**

**Ca\(^{2+}\) Buffering**

The changes of [Ca\(^{2+}\)] potentially depend as much on the Ca\(^{2+}\) buffering properties of the cell as on the fluxes of total Ca\(^{2+}\). In quiescent cells (or at low pacing rates), an increase in buffering is not expected to change diastolic [Ca\(^{2+}\)], since free [Ca\(^{2+}\)] (and not the Ca\(^{2+}\) bound to buffers) determines efflux, and this must balance influx, which is constant. At higher pacing rates, because an increase in buffering slows the rate of change of [Ca\(^{2+}\)], the Ca transient cannot decay back to baseline and end-diastolic [Ca\(^{2+}\)] will rise. Accordingly, experimentally increasing the cytoplasmic buffering power slows the rate of decay of [Ca\(^{2+}\)] and elevates [Ca\(^{2+}\)] and force in diastole. An increase in diastolic [Ca\(^{2+}\)] in hypertrophic cardiomyopathy resulting from some troponin T mutations has been attributed to this mechanism and may contribute to contractile impairment at increased heart rates in this condition.

**Sarcoplasmic Reticulum Ca-ATPase**

The greater the activity of SERCA, the faster systolic [Ca\(^{2+}\)] will decay, and, all other things being equal, the further diastolic [Ca\(^{2+}\)] will fall before the next beat and, therefore, the lower will be end-diastolic [Ca\(^{2+}\)]. Experimentally decreasing SERCA activity can (see above) increase diastolic [Ca\(^{2+}\)] and pressure as a consequence of the slowing of the decay of the Ca\(^{2+}\) transient. The increased diastolic [Ca\(^{2+}\)] will compensate for the decreased systolic efflux resulting from the smaller Ca\(^{2+}\) transient thereby maintaining Ca\(^{2+}\) flux balance. It should, however, be noted that acute inhibition of SERCA has been reported to increase [Na\(^{+}\)] and...
this can elevate diastolic [Ca\(^{2+}\)] via NCX. The origin of this increase in [Na\(^+\)] is unclear. One possibility is that the decreased amplitude of the Ca\(^{2+}\) transient will have decreased inactivation of the L-type Ca current, thereby increasing Ca\(^{2+}\) entry and thence efflux on NCX, leading to loading of the cell with Na\(^+\). Given that Na\(^+\) entry on NCX is a major component of Na\(^+\) entry into the cell,\(^{22}\) this will elevate [Na\(^+\)]. In another study, knockout of SERCA also elevated [Na\(^+\)].\(^{33}\) These knockout mice have increased L-type Ca current, possibly to compensate for the lack of SERCA. This increased Ca\(^{2+}\) influx will need to be balanced by increased efflux on NCX. The consequent increase in Na\(^+\) influx may, therefore, account for the elevation of [Na\(^+\)].

**Ryanodine Receptor**

As mentioned in a previous section, making the RyR leaky can decrease SR Ca content and thence the amplitude of the systolic Ca transient and systolic Ca\(^{2+}\) efflux. The decrease of efflux means that Ca\(^{2+}\) will accumulate in the cell, increasing diastolic [Ca\(^{2+}\)] until the increase in diastolic Ca\(^{2+}\) efflux restores total efflux to equal influx. Leaky RyRs also slow the rate constant of decay of the systolic Ca transient.\(^{24,38}\) Under normal conditions, Ca release from the SR occurs more or less synchronously, a few milliseconds after the start of depolarization, in response to the rise of [Ca\(^{2+}\)]\(_{\text{SR}}\), produced by the L-type current. Release from clusters of RyRs can be seen as calcium sparks.\(^{11}\) In contrast, after myocardial infarction, Ca sparks are observed on the falling phase of the systolic Ca transient.\(^{15}\) Increasing RyR phosphorylation and opening by overexpression of CaMKII (Ca\(^{2+}\)/calmodulin-dependent protein kinase II)-δ, also leads to the appearance of delayed calcium sparks, which will interfere with the decay of [Ca\(^{2+}\)] and relaxation.\(^{36}\) Such late sparks have also been suggested to be a more general phenomenon particularly when the initial release of Ca\(^{2+}\) from the SR is depressed.\(^{36}\) A study in hypothyroid mice has linked the appearance of late sparks to impaired systolic and diastolic function.\(^{37}\)

**Sodium-Calcium Exchange**

NCX uses the energy provided by 3 Na\(^+\) entering to pump 1 Ca\(^{2+}\) out of the cell. This stoichiometry generates an electric current\(^{23,38}\) and NCX activity is sensitive not only to the Na\(^+\) and Ca\(^{2+}\) concentration gradients but also to membrane potential; hyperpolarization increases and depolarization decreases net Ca\(^{2+}\) efflux. Depending on the ionic gradients and membrane potential, NCX can reverse direction with net Ca\(^{2+}\) influx coupled to Na\(^+\) efflux (reverse mode). At a normal resting potential, NCX works in the forward direction with Ca\(^{2+}\) efflux roughly proportional to [Ca\(^{2+}\)].\(^{17}\) It should, however, also be noted that NCX is allosterically regulated by [Ca\(^{2+}\)], thus limiting Ca\(^{2+}\) efflux at low [Ca\(^{2+}\)].\(^{39}\) For an extensive review of NCX, see the article by Blaustein and Lederer.\(^{40}\)

**Intracellular Sodium**

An increase in [Na\(^+\)], decreases the driving force available for NCX to remove Ca\(^{2+}\) from the cell and thereby increases developed force and the underlying systolic Ca transient. In rabbit ventricular myocytes, inhibition of the Na-K pump increases both [Na\(^+\)] and diastolic [Ca\(^{2+}\)].\(^{41}\) However, at least with moderate increases in [Na\(^+\)], there is often no increase in diastolic [Ca\(^{2+}\)]\(^{42,43}\) or developed force/cell length.\(^{44,46}\) While this may result partly from the low sensitivity of force and some Ca\(^{2+}\) indicators to [Ca\(^{2+}\)], it may also be explained as follows (Figure 3). In the steady state, the reduction of NCX activity will require an increase in average [Ca\(^{2+}\)] (see above). At first, this will be largely provided by an increase in systolic [Ca\(^{2+}\)] as a result of the increase in SR Ca content. Only with further reduction of NCX, perhaps because there is a limit to how much SR Ca content and thence systolic [Ca\(^{2+}\)] can increase, will diastolic [Ca\(^{2+}\)] increase appreciably.

**Plasma Membrane Ca-ATPase**

In addition to NCX, the myocyte also expresses a PMCA whose contribution to Ca\(^{2+}\) efflux is less well established.\(^{47}\) It has been argued that the PMCA is irrelevant to the control of bulk cytoplasmic [Ca\(^{2+}\)] and, instead, has a signaling function by controlling [Ca\(^{2+}\)] in micro-domains near caveolae.\(^{48}\) Work from the Bers Laboratory finds that the contribution of the PMCA to Ca\(^{2+}\) removal in a variety of species is typically <10% of that of NCX.\(^{49}\) We find a larger contribution; inhibiting NCX with Ni\(^{2+}\) leaves 25% to 33% of the Ca\(^{2+}\) removal from the cell functional in rat.\(^{16,50}\) A concern with the use of Ni\(^{2+}\) is that it may not completely inhibit NCX, but similar results are seen when NCX is stopped by removal of Na\(^+\) ions.\(^{51,52}\) The NCX-independent Ca\(^{2+}\) efflux is abolished by the nonspecific PMCA inhibitor carboxyeosin.\(^{53,54}\) A substantial role for PMCA is also suggested by work on myocytes isolated from NCX knockout mice. These animals live normally, and their ventricular myocytes have normal Ca\(^{2+}\) transients. There is no change of PMCA expression, and the myocytes maintain Ca\(^{2+}\) flux balance by decreasing Ca influx through the L-type Ca current to 20%–a level at which PMCA alone can presumably balance it.\(^{55,56}\) This suggests that PMCA makes a contribution equivalent to 25% of that of NCX in the wild type. One caveat is that, as in other studies, the rate of Ca\(^{2+}\) removal from the cell was assessed from the rate of fall of the caffeine-evoked rise of [Ca\(^{2+}\)]. The available data do not provide caffeine exposures of sufficient duration to obtain accurate measurements,\(^{56}\) and further work is required to establish the role of PMCA in the regulation of diastolic [Ca\(^{2+}\)].
Mitochondrial Ca\textsuperscript{2+} Handling

In principle, Ca\textsuperscript{2+} uptake and release from mitochondria could affect diastolic [Ca\textsuperscript{2+}]. As we have recently reviewed,	extsuperscript{6} there are conflicting reports in the literature with only some studies finding evidence in favor of beat-to-beat movements of Ca\textsuperscript{2+} into and out of mitochondria. On balance, at least in adult ventricular myocytes, while changes of mitochondrial [Ca\textsuperscript{2+}] can be observed at slow rates of stimulation,	extsuperscript{57} they disappear at higher rates questioning their importance in regulating diastolic [Ca\textsuperscript{2+}].

Ca\textsuperscript{2+} Influx Pathways During the Action Potential

The major route for Ca\textsuperscript{2+} entry during the action potential is the L-type Ca current.\textsuperscript{58} In some regions of the heart, particularly in nodal tissues, there are also contributions from the T-type Ca channel.\textsuperscript{59} The stoichiometry of NCX means that it can also contribute to Ca\textsuperscript{2+} influx during depolarization, but, under normal conditions, this is much smaller than that through the L-type channel.\textsuperscript{60} In heart failure, the increase in [Na\textsuperscript{+}], will increase influx through NCX,\textsuperscript{61} and it is possible that the magnitude of Ca\textsuperscript{2+} influx through NCX may have been underestimated due to making measurements at slow rates where [Na\textsuperscript{+}] is decreased.

Many studies have investigated the effects on systolic [Ca\textsuperscript{2+}] of maneuvers that alter the L-type Ca current. Inspection of most data shows little effect on diastolic levels,\textsuperscript{6,95} but the majority of experiments were performed at slow rates or used the whole-cell patch clamp technique. We found that decreasing the L-type Ca current with cadmium in cells where diastolic [Ca\textsuperscript{2+}] was elevated reduced diastolic [Ca\textsuperscript{2+}].\textsuperscript{14} From first principles, one would expect 2 opposing effects.\textsuperscript{64} (1) Increased L-type Ca current will increase Ca\textsuperscript{2+} influx per unit time thereby requiring an increased average [Ca\textsuperscript{2+}], to balance it. Depending on the conditions, this may be achieved by increased systolic or diastolic [Ca\textsuperscript{2+}], (2) The increase in L-type current will increase Ca\textsuperscript{2+} release from the SR, increasing systolic Ca, thereby contributing to the elevated average without the need to increase diastolic. This latter effect, however, is limited as it is impossible to release >100% of SR content. It should also be noted that, at least under some conditions, increasing L-type Ca current does not increase SR content.\textsuperscript{64} Additionally, the increase in systolic [Ca\textsuperscript{2+}], will increase the time taken for [Ca\textsuperscript{2+}] to decay back to baseline, increasing the tendency for diastolic [Ca\textsuperscript{2+}], to rise at shorter pacing intervals (higher heart rates). Quantitative considerations will determine whether the increase in systolic efflux is sufficient to balance the increase in influx or, alternatively, whether elevated diastolic [Ca\textsuperscript{2+}], occurs.

Background Ca\textsuperscript{2+} Entry Mechanisms

In the absence of stimulation, resting [Ca\textsuperscript{2+}] is of the order of 100 nmol/L indicating that some kind of background Ca\textsuperscript{2+} entry pathway must exist to balance Ca\textsuperscript{2+} efflux on NCX. Such a pathway accounts for the fact that, even in a quiescent cell, after being emptied with caffeine, the SR can be refilled by a mechanism that requires extracellular Ca\textsuperscript{2+}.\textsuperscript{65} As mentioned above, Ca\textsuperscript{2+} waves can occur in cells held at a fixed membrane potential,\textsuperscript{23} again indicating an influx pathway to balance efflux on NCX during the waves. The magnitude of this influx is roughly proportional to external Ca\textsuperscript{2+} concentration in the range ≤5 mmol/L.\textsuperscript{23} Subsequent work, examining the effects on resting [Ca\textsuperscript{2+}] of abruptly removing external Ca\textsuperscript{2+}, provided an estimate for the background Ca\textsuperscript{2+} influx of the order of 2 to 6 µmol/L per s in rat ventricular myocytes.\textsuperscript{56} A recent study estimated Ca\textsuperscript{2+} influx from measurements of average [Ca\textsuperscript{2+}] (see above) and found a value of about 4 µmol/L per s.\textsuperscript{14} These values compare to an entry on each action potential via the L-type Ca current of the order of 5 to 10 µmol/L. Therefore, at normal heart rates (in a rat) of 5 s\textsuperscript{-1}, the background influx will be of the order of 10% of that carried by the L-type current. As regards the mechanism of this influx, one study identified a Ca\textsuperscript{2+} entry mechanism that increased on hyperpolarization of the surface membrane and was blocked by the relatively nonspecific agent gadolinium (Gd\textsuperscript{3+}).\textsuperscript{57} It is, therefore, important to consider the identity of this flux.

Connexin Hemichannels

One Ca\textsuperscript{2+} flux inhibited by Gd\textsuperscript{3+} is that carried by connexins.\textsuperscript{58} The majority of connexins are found as pairs, made up of 2 hemichannels, one in each of the 2 cell membranes at the intercalated discs. These allow current to flow between cells. However, some connexins are present as hemichannels in the surface membrane of a single cell\textsuperscript{90,70} and may, therefore, provide a route for Ca\textsuperscript{2+} entry. Recent work has suggested that this entry may be increased in experimental cardiomyopathy induced by plakophilin-2 deficiency.\textsuperscript{71}

Transient Receptor Potential Channels

Transient receptor potential (TRP) channels are also sensitive to Gd\textsuperscript{3+}, and considerable work has investigated their role in the heart. Knockout of TRPV2 decreases the amplitude of the systolic Ca transient and contraction.\textsuperscript{72} The compound probenecid, which activates TRPV2, was also shown to increase contractility,\textsuperscript{73} and a small trial has shown that this compound improves cardiac function in patients with heart failure.\textsuperscript{74} It should, however, be noted that probenecid has other actions including inhibiting organic anion transporters.\textsuperscript{75} Furthermore, inhibition of TRPV4 decreases SR Ca release.\textsuperscript{76} The cardiomyopathy found in the mdx mouse model of muscular dystrophy is associated with elevated diastolic [Ca\textsuperscript{2+}], which can be blocked by Gd\textsuperscript{3+}, and has been attributed to Ca\textsuperscript{2+} entry via TRPC channels.\textsuperscript{77} Similar results were found for the experimental myopathy produced by infusion of isoproterenol.\textsuperscript{78} Further evidence suggesting a role for TRP channels in contributing to setting diastolic [Ca\textsuperscript{2+}] comes...
from the observation that knocking out both TRPC1 and TRPC4 in mice decreased diastolic \([\text{Ca}^{2+}]\).\textsuperscript{79} The recent synthesis of specific antagonists of TRPC channels\textsuperscript{80} and an agonist\textsuperscript{81} should make it possible to study the role of these channels more precisely.

TRP channels have also been implicated in the influx of \(\text{Ca}^{2+}\) into the cell activated by emptying the SR, so called store-operated \(\text{Ca}^{2+}\) entry, and in the HL-1 cell line, this has been suggested to contribute to resting \([\text{Ca}^{2+}]\).\textsuperscript{82} One issue is that much of the evidence for a role of store-operated channels in cardiac tissue comes from work on cultured or neonatal cells,\textsuperscript{83,84} and these may not be representative of adult myocytes. Some recent articles have, however, reported store-operated \(\text{Ca}^{2+}\) entry into adult mouse ventricular myocytes\textsuperscript{85} with the fluxes being inhibited by Gd\textsuperscript{3+}.\textsuperscript{86,87} In many tissues, store-operated calcium entry is produced by a combination of the endoplasmic reticulum \(\text{Ca}^{2+}\) sensor STIM1 (stromal interaction molecule 1) and the surface membrane channel Oral1 (see the article by Qiu and Lewis\textsuperscript{88} for review). Overexpression of STIM1 in mouse heart increases diastolic \([\text{Ca}^{2+}]\), as a result of increased \(\text{Ca}^{2+}\) entry into the cell and increased leak from the SR.\textsuperscript{89} It should, however, be noted that STIM1 has also been reported to interact with PLN and thereby control SERCA.\textsuperscript{90}

Finally, some TRP channels and connexins transport Na\textsuperscript{+} in addition to \(\text{Ca}^{2+}\) and, by altering \([\text{Na}^{+}]\), could affect \([\text{Ca}^{2+}]\) indirectly via NCX. All in all, it is clear that more work is required to characterize the contribution of TRPs, connexins, and as yet unidentified mechanisms to the background \(\text{Ca}^{2+}\) influx

### PHYSIOLOGICAL FACTORS AFFECTING DIASTOLIC \([\text{Ca}^{2+}]\)

#### Heart Rate

Increasing the rate of stimulation increases diastolic \([\text{Ca}^{2+}]\) in ventricular trabeculae\textsuperscript{91} and isolated myocytes.\textsuperscript{14,92–95} It is important to note that the \(\text{Ca}^{2+}\) indicators used to measure \([\text{Ca}^{2+}]\), buffer \([\text{Ca}^{2+}]\), to some degree and potentially exaggerate the effects of increased frequency. It would be useful to repeat these experiments using as low concentrations of Ca indicators as possible. Of course the fact that, even in the absence of indicators, increasing stimulation rate increases diastolic force\textsuperscript{96} and decreases cell length\textsuperscript{92} means that excessive buffering cannot account for all the effects.

There are at least 2 possible explanations for the frequency-dependent increase in diastolic \([\text{Ca}^{2+}]\) (Figure 4). One is that increasing frequency increases \([\text{Na}^{+}]\)\textsuperscript{41,97,98} and, as discussed above, decreases NCX activity, requiring an increase in average \([\text{Ca}^{2+}]\) to maintain flux balance. This explanation is consistent with the parallel increase in \([\text{Na}^{+}]\) and diastolic \([\text{Ca}^{2+}]\).\textsuperscript{99} Two arguments, however, suggest that Na-independent mechanisms may also be involved. (1) The increase in diastolic \([\text{Ca}^{2+}]\) can occur abruptly on increase in rate,\textsuperscript{94} much faster than the presumed increase in at least global \([\text{Na}^{+}]\), (2) In the NCX knockout mouse (where changes of \([\text{Na}^{+}]\) would not be expected to increase \([\text{Ca}^{2+}]\)), the effects of rate on diastolic \([\text{Ca}^{2+}]\) are similar to those in wild type.\textsuperscript{65} As discussed above, and in Figure 4, this Na-independent factor is likely to be the need for the increase in \(\text{Ca}^{2+}\) influx to be balanced by increased efflux and, therefore, elevated average \([\text{Ca}^{2+}]\). Why does diastolic \([\text{Ca}^{2+}]\), increase at higher rates? It might be thought that balance could occur simply by the increased frequency of Ca transients resulting in more systolic efflux. This, however, ignores that (1) the Ca transient cannot decay back to equilibrium before the next beat, resulting in end-diastolic \([\text{Ca}^{2+}]\), rising (2) there is less time for NCX Ca removal from the cell per beat. Finally, in species with a negative force-frequency relationship (and humans with heart failure\textsuperscript{100}), the systolic Ca transient decreases at higher rates presumably reducing the systolic efflux per beat. Both Na-dependent and independent mechanisms may contribute. The increase in \(\text{Ca}^{2+}\) entry at higher rates demands increased \(\text{Ca}^{2+}\) efflux for flux balance. This will require an increase in average \([\text{Ca}^{2+}]\). The elevated \([\text{Na}^{+}]\), will decrease NCX activity thereby requiring a greater increase in average \([\text{Ca}^{2+}]\).

#### β-Adrenergic Stimulation

Physiologically, changes of β-adrenergic stimulation are the main cause of changes of heart rates. The effects of β-adrenergic stimulation will, therefore, be a combination of those of rate, discussed above, as well as direct effects. The latter include an increase in both Ca entry through the L-type Ca current and of SERCA activity (see above—via phosphorylation of PLN). The expected effects of these have been discussed earlier. In brief, the level of diastolic \([\text{Ca}^{2+}]\) will be determined by the net effects of β-adrenergic stimulation on Ca entry via the L-type current and on the shape and size of the Ca transient, which determine NCX removal. β-Adrenergic stimulation increases influx via \(I_{\text{Ca,L}}\) which is partly balanced by a larger Ca transient amplitude and, therefore, greater systolic efflux via NCX. This latter effect is curtailed, however, by the accelerated decay of the Ca transient resulting from greater SERCA activity.\textsuperscript{101} Accordingly, in rat ventricular myocytes, studied at constant rate, β-adrenergic stimulation increases diastolic \([\text{Ca}^{2+}]\).\textsuperscript{94} It should also be noted that β-adrenergic stimulation phosphorylates phospholemman, increasing Na-K pump activity, decreasing \([\text{Na}^{+}]\), and thereby increasing the driving force for NCX-mediated \(\text{Ca}^{2+}\) efflux. This will decrease the level of average \([\text{Ca}^{2+}]\), required to balance the increased \(\text{Ca}^{2+}\) influx.

The Ca transient and accompanying NCX removal could also be affected by changes in \(\text{Ca}^{2+}\) buffering
arising from PKA-dependent phosphorylation of cardiac troponin I and PLN during β-adrenergic stimulation. The effect on cardiac troponin I will lower the affinity for Ca²⁺ binding and, except at high levels of [Ca²⁺], would be expected to decrease Ca²⁺ buffering and accelerate the decay of the Ca transient. Conversely, phosphorylation of PLN will increase affinity and increase buffering and should slow the decay. Experiments on mouse ventricular myocytes found that these effects were balanced such that there was no net effect on Ca²⁺ buffering.¹⁰¹ Further work is required to investigate the effects of β-adrenergic stimulation in a wider range of conditions.

**CLINICAL ASPECTS OF ABNORMAL DIASTOLIC FUNCTION**

The previous sections have reviewed the fundamental mechanisms that regulate diastolic [Ca²⁺]. Before turning to the changes of calcium cycling that occur in heart failure, it is important to set these in a clinical context.

**Diastolic Dysfunction**

Ventricular filling in diastole relies on both a compliant ventricle and a pressure gradient between the left atrium and left ventricle (LV). In the early phase of diastole, active ventricular relaxation helps to generate this gradient by actively sucking blood into the ventricle via elastic recoil. This active phase is myocyte dependent and relies on the rapid decline in [Ca²⁺] at the beginning of diastole, leading to dissociation of the thick and thin filaments. In the subsequent, passive phases of diastole, the pressure gradient distends the ventricle.¹⁰² While this phase depends heavily on passive properties of the myocardium including wall thickness and fibrosis, it is also determined by diastolic [Ca²⁺] by setting the baseline myofilament activation and thus tension. Both active and passive processes require the heart to be sufficiently relaxed and compliant to fill with blood. During exercise, heart rate rises and diastolic interval decreases. Here, LV filling is maintained by increasing transmitral flow via an increase in pressure gradient. In the healthy heart, this gradient is generated by enhanced elastic recoil to reduce LV pressure in early diastole, without significantly changing left atrium pressure.¹⁰³

Slowing of relaxation leads to diastolic dysfunction, and this is particularly pronounced during dynamic exercise with exercise intolerance—a frequent presenting feature of heart failure. Consequently, LV diastolic pressure increases, and filling can only be achieved by an increase in left atrium pressure, resulting in pulmonary congestion, breathlessness, and effort intolerance.¹⁰⁴ More advanced stages of diastolic dysfunction display elevated filling pressures at rest.

Diastolic dysfunction is frequently observed alongside systolic dysfunction in heart failure with reduced ejection fraction (HFrEF). There are multiple mechanisms for slowed relaxation in HFrEF, including abnormalities in Ca²⁺ cycling (including reduced Ca uptake via SERCA)¹⁰⁵ and changes in diastolic [Ca²⁺], (see below), increased extracellular collagen,¹⁰⁶ increased myofilament crossbridge interactions due to metabolic changes independent of Ca²⁺,¹⁰⁷⁻¹⁰⁹ and loss of elastic recoil (due to failure of elastic compression in systole).¹⁰⁹ Importantly, however, about half of patients with heart failure have diastolic dysfunction but a normal ejection fraction or heart failure with preserved ejection fraction (HFpEF; for review, see the article by Pfeffer et al¹¹⁰). The increase in chamber stiffness and slowed relaxation observed in HFpEF causes a rise in LV filling pressures, which, when sufficiently high, results in the heart failure syndrome.¹¹¹ It is increasingly clear that HFpEF is not a condition of diastolic dysfunction alone and some impairment in systolic function is present at rest and
becomes more prominent during exercise.\textsuperscript{112} This systolic impairment may further exacerbate diastolic dysfunction because contractile impairment modifies the restoring forces that drive early diastolic recoil.\textsuperscript{113} Additionally, HFpEF is associated with a constellation of comorbidities such as diabetes mellitus, obesity, hypertension, aging, and kidney disease. Consequently, HFpEF is accompanied by systemic changes, including inflammation and endothelial dysfunction, tissue fibrosis, microvascular dysfunction and ischemia, and multiorgan impairment such as renal failure and sarcopenia. These contribute both to the diastolic impairment and the overall clinical phenotype.\textsuperscript{115} In spite of its complexity, the inherent defects underlying diastolic dysfunction can be broadly grouped into 2 classes: (1) external to the cardiac myocyte and (2) resulting from impaired myocyte function.

**Myocyte-Independent Mechanisms**

The extracellular matrix is a major determinant of myocardial stiffness, and increases in interstitial fibrosis and collagen are observed in HFpEF,\textsuperscript{114} as well as being part of the aging process.\textsuperscript{115} In addition to increasing stiffness,\textsuperscript{116} expansion of the extracellular matrix in HFpEF is associated with increased mortality and rates of hospitalization.\textsuperscript{117} It has also been proposed that elevations in LV filling pressure may result from increased extrinsic restraint on the heart,\textsuperscript{118} for example, in the obese phenotype of HFpEF where epicardial fat may cause mechanical compression of the heart, as well as exerting paracrine effects.\textsuperscript{119,120} Finally, it is worth noting that LV geometry itself may impact on diastolic function. Concentric hypertrophy is commonly observed clinically in HFpEF,\textsuperscript{121} particularly in patients with systemic arterial hypertension, and results from both expansion of the interstitium and myocyte hypertrophy.\textsuperscript{106,122} Here, an increase in wall thickness elevates stiffness and contributes to the diastolic impairment.\textsuperscript{123,124}

**Myocyte-Dependent Mechanisms**

Dysfunctional relaxation and higher passive stiffness in HFpEF is present at the level of the cardiac myocyte.\textsuperscript{125} Traditionally, diastolic dysfunction has been attributed to increased stiffness secondary to gross concentric hypertrophy (typically caused by hypertension),\textsuperscript{126} which is also present in isolated myocytes.\textsuperscript{127} However, a significant proportion of HFpEF patients do not have LV hypertrophy, and severity of hypertrophy does not closely correlate with diastolic dysfunction.\textsuperscript{128} Instead, the bulk of this increase in resting tension can be explained at the sarcomere.

The giant molecular spring titin, which spans the Z disk to M band, is a major determinant of passive tension by providing recoil in early diastole and resistance to stretch in late diastole.\textsuperscript{129,130} Its properties can be directly modified by phosphorylation (by protein kinases A and G, and CaMKII, which reduce tension)\textsuperscript{129–131} and oxidative modification via disulphide bonds\textsuperscript{132} and S-glutathionylation.\textsuperscript{133} As such, in addition to changes in its expression, posttranslational modifications in titin allow for dynamic changes in cellular and diastolic stiffness, which are implicated in the pathophysiology of HFpEF. Finally and intriguingly, the titin N2BA isoform exhibits a small [Ca\textsuperscript{2+}]-dependent increase in stiffness.\textsuperscript{134,135} Although this may add further importance to the role of diastolic Ca\textsuperscript{2+} in diastolic dysfunction, the significance of this finding in vivo has not yet been established.

At the sarcomere level, there is also evidence implicating the actin-myosin filaments in HFpEF. Relaxation of these depends on both diastolic [Ca\textsuperscript{2+}] (see subsequent sections) and their sensitivity to Ca\textsuperscript{2+}. Increased myofilament Ca\textsuperscript{2+} sensitivity secondary to hypophosphorylation of cardiac troponin I has been reported in HFpEF.\textsuperscript{126} Furthermore, abnormally high myofilament Ca sensitivity also contributes to the diastolic dysfunction observed in hypertrophic cardiomyopathy caused by sarcomeric gene mutations.\textsuperscript{122,128} Accordingly, the increase in resting tension in HFpEF myocytes has been linked with low PKG (protein kinase G) levels, which may impair relaxation by reducing phosphorylation of titin, cardiac troponin I, and PLN.\textsuperscript{133,140} A role for defective CaMKII phosphorylation of titin has also been proposed.\textsuperscript{131} In conclusion, although other factors may contribute to impaired diastolic function, it is important to consider the role of abnormalities in Ca\textsuperscript{2+} signaling.

**DIASTOLIC [Ca\textsuperscript{2+}] IN HEART FAILURE**

Does diastolic [Ca\textsuperscript{2+}] change in heart failure? We will first consider data from animal and human studies where systolic function is also impaired before moving on to HFpEF.

**Heart Failure With Reduced Ejection Fraction**

The decreased systolic Ca transient in heart failure may result in large part from a decrease in SR Ca\textsuperscript{2+} content caused by one or more of decreased SERCA activity, leaky RyRs, or increased NCX activity (see the article by Bers\textsuperscript{105} for review). As far as diastolic [Ca\textsuperscript{2+}] is concerned, measurements on ventricular strips from patients with heart failure found increases in diastolic force and [Ca\textsuperscript{2+}], which were most obvious at higher stimulation frequencies.\textsuperscript{141} Ca transients in cells isolated from patients had a smaller amplitude and also slowed decay,\textsuperscript{12} which would be expected to increase diastolic [Ca\textsuperscript{2+}]. A subsequent study found little elevation of diastolic [Ca\textsuperscript{2+}] or force\textsuperscript{66} but pointed out that the lack of sensitivity of the Ca\textsuperscript{2+} indicator used may have made it hard to resolve changes of diastolic [Ca\textsuperscript{2+}]. Experiments on myocytes from patients with heart failure, using more sensitive fluorescent indicators (fluo-3 and fura-red), demonstrated an increase...
in diastolic $[\text{Ca}^{2+}]$, with increasing rate,\textsuperscript{142} but no control data were available. Increasing the rate of stimulation increased diastolic force in ventricular muscle strips from patients with heart failure but not controls.\textsuperscript{143} In a rabbit model of aortic insufficiency/restriction, the amplitude of the systolic Ca transient decreased to about 70% of control with no change of diastolic $[\text{Ca}^{2+}]$.\textsuperscript{144} It should, however, be noted that the experiments were performed at a slow rate (0.5 Hz). Another study on rabbit myocytes found that pressure and volume overload–induced heart failure increased diastolic $[\text{Ca}^{2+}]$.\textsuperscript{146} and, in contrast to much of the other work discussed here, this was unaffected by stimulation frequency. Finally, a study of right side heart failure (induced in rats with monocrotaline) showed a stimulated frequency.\textsuperscript{147} It should, however, be noted that the experiments were performed at a slow rate (0.5 Hz). Another study on rabbit myocytes found that pressure and volume overload–induced heart failure increased diastolic $[\text{Ca}^{2+}]$.\textsuperscript{148} and, in contrast to much of the other work discussed here, this was unaffected by stimulation frequency. Finally, a study of right side heart failure (induced in rats with monocrotaline) showed a tendency to increased diastolic $[\text{Ca}^{2+}]$, particularly at elevated stimulation frequencies.\textsuperscript{149} Further complication is added by reports of a decrease in diastolic $[\text{Ca}^{2+}]$, in a sheep tachypacing model of heart failure, albeit studied at low stimulation rates.\textsuperscript{147} Interestingly, this was accompanied by a decrease in the L-type Ca current, which would be expected to decrease average $[\text{Ca}^{2+}]$, perhaps contributing to the decrease in diastolic $[\text{Ca}^{2+}]$. Unchanged diastolic $[\text{Ca}^{2+}]$ was found in ventricular myocytes from tachypaced dogs, but this also used low stimulation rates and whole-cell patch clamp.\textsuperscript{148} Decreased diastolic $[\text{Ca}^{2+}]$, has also been found in a ferret aortic banding model of hypertrophy, again at low stimulation rates.\textsuperscript{149}

An important clinical situation that produces heart failure and depressed myocardial contractility is sepsis. Cecal ligation and puncture in the rat slowed the decay of the Ca transient; this was attributed to increased frequency of Ca sparks and accompanied by decreased systolic and increased diastolic $[\text{Ca}^{2+}]$.\textsuperscript{150} In another study on rats, using lipopolysaccharide administration, septic cardiomyopathy slowed the decay of $[\text{Ca}^{2+}]$.\textsuperscript{151} This was suggested to result from decreased activity of NCX and PMCA. This is surprising because these sarcolemmal transporters make only a small contribution (compared with SERCA) to the decay of the systolic Ca transient in small animals. In contrast, lipopolysaccharide administration in mice also slowed the decrease in the Ca transient, but this was associated with decreased SERCA activity due to sulfonation.\textsuperscript{152} This was accompanied by a small decrease in diastolic $[\text{Ca}^{2+}]$ over the range of 1 to 6 Hz, the explanation for which is not clear.

Although it is not easy to draw conclusions from the above work on patients and animal models with HFrEF, it does appear that in the majority of studies where physiological rates have been studied, there is a frequency-dependent increase in diastolic $[\text{Ca}^{2+}]$ and force. More work is needed at physiological rates to characterize this.

**Heart Failure With Preserved Ejection Fraction**

A major issue with studying HFrEF in animals has been the difficulty of producing an appropriate model.\textsuperscript{153,154} Work in 2 articles has developed potential models of HFrEF by banding the aorta in rats. In one, ventricular myocytes displayed an increase in both diastolic $[\text{Ca}^{2+}]$, and the amplitude of the Ca transient. These effects were attributed, at least in part, to increased Ca$^{2+}$ leak through the RyR (seen as increased Ca spark frequency) and decreased NCX activity.\textsuperscript{155} In the other, although the animals and isolated ventricular trabeculae had impaired diastolic function, isolated myocytes, taken from the same hearts, showed lower diastolic $[\text{Ca}^{2+}]$, and shortening, suggesting that the main cause of mechanical dysfunction involved passive mechanisms rather than Ca$^{2+}$ handling.\textsuperscript{156} Modeling studies have pointed out that the maintained ejection fraction in HFrEF could be achieved despite a decrease in systolic $[\text{Ca}^{2+}]$, due to the compensatory effect of concentric ventricular hypertrophy.\textsuperscript{157} Another, recently developed, model of HFrEF is that of an inbred rat with a hypertrophic heart. This has increased diastolic and systolic $[\text{Ca}^{2+}]$, accompanied by an increase in the L-type Ca current.\textsuperscript{158} It is, therefore, possible that the increase in both diastolic and systolic $[\text{Ca}^{2+}]$, augments Ca$^{2+}$ efflux to compensate for the increased influx. In the absence of measurements, however, it is impossible to exclude a contribution from effects of $[\text{Na}^{+}]$, mediated via NCX. Interestingly, the rate of decay of the Ca transient was accelerated arguing against decreased SERCA activity.

Kidney disease is a risk factor for HFrEF, and this has been modeled experimentally by removing 80% of renal tissue resulting in prolonged ventricular relaxation and elevated end-diastolic pressure. Early work found elevated diastolic $[\text{Ca}^{2+}]$, attributed to altered NCX possibly due to increased $[\text{Na}^{+}]$.\textsuperscript{159} A subsequent study\textsuperscript{159} showed a slowing of the rate of decay of both cell shortening and the systolic Ca transient but no effect on the level of either systolic or diastolic $[\text{Ca}^{2+}]$. The experiments were, however, performed at a slow rate. This study also found that acute administration of the NCX inhibitor SEA0400 accelerated the decay of the systolic Ca transient, but the mechanism was unclear. Another study from this group found that in vivo administration of another NCX inhibitor (ORM-11035) also accelerated relaxation\textsuperscript{160}—a result consistent with studies on the Dahl salt-sensitive rat where the improved relaxation produced by NCX inhibition was attributed to an effect on fibroblasts, decreasing fibrosis.\textsuperscript{161}

As mentioned above, diastolic dysfunction is clinically observed in diabetes mellitus. Work on a streptozotocin rat model, with normal systolic and impaired diastolic function, found a decrease in the rate constant of decay of the systolic Ca transient due to decreased SERCA activity.\textsuperscript{162} Another study using the same model observed a slowing of decay, but this was accompanied by a fall not only of systolic but also diastolic $[\text{Ca}^{2+}]$ during stimulation at 1 Hz.\textsuperscript{163} It is unclear why diastolic $[\text{Ca}^{2+}]$ should decrease. Some studies have found decreased L-type
Ca current, which may decrease average [Ca$^{2+}$]. In addition, the slowing of decay of the Ca transient will increase average [Ca$^{2+}$] thereby allowing a lower diastolic [Ca$^{2+}$] as long as there is sufficient time for [Ca$^{2+}$] to fall in diastole. A similar study found a decrease in resting [Ca$^{2+}$] in unstimulated cells. This argues for alterations of background Ca$^{2+}$ influx or NCX/PMCA. It should, however, be noted that there is evidence that the depression of contractility in the streptozotocin rat may be independent of changes of [Ca$^{2+}$]. Metabolic dysregulation is also associated with development of HFpEF. A recent study found that the ZSF-1 obese rat had elevated diastolic but similar systolic [Ca$^{2+}$] compared with controls. Mitochondrial [Ca$^{2+}$] was also elevated and suggested to partly compensate by stimulating metabolism but also to result in adverse consequences of mitochondrial overload. This article also reported a decrease in diastolic [Ca$^{2+}$] with increasing stimulation rate—a result that differs from other studies reviewed here. Atria from the ZSF-1 obese rat also show impaired function but no effect on either the rate constant of decay of the Ca transient or diastolic [Ca$^{2+}$] was observed. Finally, a recent publication has introduced a mouse model of HFpEF using a combination of high-fat diet and nitrosa-

codiopiazonic acid plus ryanodine, there was an eleva-

tion of diastolic force, and this was abolished by the contractile uncoupler butanedione monoxime suggesting that it resulted from myofilament activation. When SR function was inhibited by the SERCA inhibitor cyclopiazonic acid plus ryanodine, there was an elevation of diastolic force, which was much greater in those preparations that had previously developed significant diastolic force with raised frequency.

Finally, hypertrophic cardiomyopathy can also result in a heart failure syndrome. While this is a separate disease entity to HFpEF, it also leads to diastolic dysfunction. Hypertrophic cardiomyopathy is often an inherited condition that can result from mutations in the sarcomeric proteins, which make up the thick and thin filaments, (reviewed in ). Many of these mutations increase the sensitivity of the myofilaments for [Ca$^{2+}$]. This, alone, would increase diastolic force, but, in addition, the increase in Ca$^{2+}$ buffering slows the decay of [Ca$^{2+}$], elevating end-diastolic [Ca$^{2+}$] and, therefore, diastolic force/pressure.

How Does Diastolic [Ca$^{2+}$] Increase in Heart Failure?

Although, as discussed above, there is considerable variation between studies, the consensus appear to be that diastolic [Ca$^{2+}$] increases in heart failure. There are at least 2 (nonexclusive) explanations for this. (1) As discussed in an earlier section, any decrease in the systolic Ca transient will decrease Ca$^{2+}$ efflux during systole, requiring a compensatory increase in diastolic [Ca$^{2+}$]. (2) Another explanation is provided by the increase in [Na$^+$], commonly observed in heart failure, which will decrease Ca$^{2+}$ efflux on NCX, thereby requiring an increase in average [Ca$^{2+}$], which may, in part, be provided by increased diastolic [Ca$^{2+}$]. Consistent with this, elevation of [Na$^+$] by inhibition of the sodium pump increased diastolic force at elevated stimulation rates. Further evidence linking NCX to diastolic function came from work on ventricular strips from failing human hearts showing that the greater the expression of NCX, the better the diastolic function. The increase in diastolic force and [Ca$^{2+}$] can also be attenuated by the drug ranolazine—a blocker of the late sodium current that decreases [Na$^+$]. Similarly, work on rats found that ranolazine reversed the diastolic impairment produced by the anticancer drug doxorubicin. In canine myocytes, experimental ischemic heart failure increased diastolic [Ca$^{2+}$] at elevated rates, and this was normalized by ranolazine or tetrodotoxin. Work on mice found that overexpressing CaMKII decreased systolic and increased diastolic force. The decrease of systolic [Ca$^{2+}$] has been attributed to excessive phosphorylation of RyRs leading to diastolic Ca$^{2+}$ leak as evidenced by increased Ca$^{2+}$ spark frequency. Again, these effects were reversed by ranolazine thereby linking them to changes of [Na$^+$].

As mentioned in an earlier section, a different explanation of elevated diastolic [Ca$^{2+}$] has been suggested in the cardiomyopathy observed in the mdx mouse—a model of Duchenne muscular dystrophy. Here, the elevated diastolic [Ca$^{2+}$] is normalized by Gd$^{3+}$ suggesting that it originates from Ca$^{2+}$ entry through TRP channels.

Differences of Ca$^{2+}$ Handling in HFrEF and HFpEF: a Role for NCX and [Na$^+$]?  

An unresolved question concerns the cellular mechanisms responsible for the difference in systolic function between HFrEF and HFpEF. Figure 5 shows a speculative hypothesis. For the sake of argument, we will assume that it results from differences in Ca$^{2+}$ signaling and that, in both cases, there is a combination of increased NCX, leaky RyR, and decreased SERCA activity resulting in decreased SR Ca content and thence the amplitude of the systolic Ca transient and systolic function. The decreased systolic
Efflux will require an increase in diastolic efflux so increasing diastolic \([Ca^{2+}]_i\). These changes could, therefore, account for HFrEF (Figure 5A). The rise of \([Na^+]_i\) often seen in heart failure will slow NCX and, if sufficient, will overcome the effects of the other changes thereby maintaining SR Ca content and systolic \([Ca^{2+}]_i\), at control levels. Diastolic \([Ca^{2+}]_i\) will be increased to maintain \([Ca^{2+}]_i\) efflux despite the inhibited NCX. The combination would, therefore, produce an HFrEF phenotype (Figure 5B). It is, therefore, possible that the changes of \([Ca^{2+}]_i\) cycling that underlie HFrEF and HFpEF are qualitatively identical but that, in HFpEF, the increase in \([Na^+]_i\) dominates over the other changes. Clearly, experimental studies are required to see whether this simplistic hypothesis has any validity.

**CONCLUSIONS**

Control of diastolic calcium concentration is essential for normal cardiac function. As we have discussed, this regulation depends on precise balance between influx and efflux. However, there are still major uncertainties about how this is achieved. In particular, more work is required to investigate the role of the PMCA, as well as the nature of the background \([Ca^{2+}]_i\) influx. It is essential that studies are performed at physiological heart rates. It is also important to characterize the alterations of \([Ca^{2+}]_i\) signaling that occur in heart failure and how they may differ in failure with preserved compared with reduced ejection fraction.
8. Barcenas-Ruiz L, Wier WG. Voltage dependence of intracellular [Ca\(^{2+}\)] transients in guinea pig ventricular myocytes. Circ Res. 1987;61:148–154. doi: 10.1161/01.res.61.1.148

9. Bassani JW, Yuan W, Bers DM. Fractional SR Ca release is regulated by trigger Ca and SR Ca content in cardiac myocytes. Am J Physiol. 1996;270:C1313–C1319. doi: 10.1152/ajpcell.1996.270.6.C1313

10. Trafford AW, Díaz ME, Eisner DA. Stimulation of Ca-induced Ca release only transiently increases the systolic Ca transient: Measurements of Ca fluxes and sarcoplasmic reticulum Ca. Cardiovasc Res. 1998;37:710–717. doi: 10.1016/s0009-9266(98)00266-6

11. Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. Science. 1993;262:740–744. doi: 10.1126/science.262.5137.740

12. Allen DG, Eisner DA, Orchard CH. Characterization of oscillations of intracellular Ca concentration in ferret ventricular muscle. J Physiol. 1984;352:113–128. doi: 10.1113/jphysiol.1984.sp015281

13. Rios E. The cell boundary theorem: a simple law of the control of cytosolic calcium concentration. J Physiol. 2010;6081–84. doi: 10.1007/s12576-009-0069-2

14. Sankaranarayanan R, Kistamas K, Greensmith DJ, Venetucci LA, Eisner DA. Sarcoplasmic [Ca\(^{2+}\)] regulates diastolic levels in rat ventricular myocytes. J Physiol. 2017;595:545–555.

15. Litwin SE, Zhang D, Bridge JH. Dysynchronous Ca\(^{2+}\) sparks in myocytes from infarcted hearts. Circ Res. 2000;87:1040–1047. doi: 10.1161/01.res.87.11.1040

16. Negretti N, O’Neill SC, Eisner DA. The effects of inhibitors of sarcoplasmic reticulum function on the systolic Ca\(^{2+}\) transient in rat ventricular myocytes. J Physiol. 1992;453:591–608. doi: 10.1113/jphysiol.1992.sp019246

17. Barcenas-Ruiz L, Beuckelmann DJ, Wier WG. Sodium-calcium exchange in heart: membrane currents and changes in [Ca\(^{2+}\)]. Science. 1987;238:1720–1722. doi: 10.1126/science.3686010

18. Trafford AW, Díaz ME, Negretti N, Eisner DA. Enhanced Ca\(^{2+}\) current and decreased Ca\(^{2+}\) efflux restore sarcoplasmic reticulum Ca\(^{2+}\) content after depletion. Circ Res. 1997;81:477–484. doi: 10.1161/01.res.81.4.477

19. Shannon TR, Wang F, Puglisi J, Weber C, Bers DM. A mathematical treatment of integrated Ca dynamics within the ventricular myocyte. Biophys J. 2004;87:3351–3371. doi: 10.1529/biophysj.104.047449

20. Bennett DL, O’Neill SC, Eisner DA. Strophanthidin-induced gain of Ca\(^{2+}\) occurs during diastole and not systole in guinea-pig ventricular myocytes. Pflugers Arch. 1999;437:731–736. doi: 10.1007/s004240050639

21. Mechmann S, Pott L. Identification of Na-Ca exchange current in single cardiac myocytes. Nature. 1986;321:967–969. doi: 10.1038/321967a0

22. Kass RS, Lederer WJ, Tsien RW, Weingart R. Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres. J Physiol. 1978;281:187–208. doi: 10.1113/jphysiol.1978.sp021416

23. Díaz ME, Trafford AW, O’Neill SC, Eisner DA. Measurement of sarcoplasmic reticulum Ca\(^{2+}\) and sarcolemmal Ca\(^{2+}\) fluxes in isolated rat ventricular myocytes during spontaneous Ca\(^{2+}\) release. J Physiol. 1997;501(pt 1):1–16. doi: 10.1111/j.1469-7793.1997.tb17331.x

24. Trafford AW, Díaz ME, Sibbing GC, Eisner DA. Modulation of CICR by effects on systolic Ca\(^{2+}\): measurements of sarcoplasmic reticulum and sarcolemmal Ca\(^{2+}\) fluxes in rat ventricular myocytes. J Physiol. 2000;522 (pt 2):259–270. doi: 10.1111/j.1104-8626.2000.tb02059.x

25. Greensmith DJ, Galli GL, Trafford AW, Eisner DA. Direct measurements of SR free Ca reveal the mechanism underlying the minimal effects of RYR potentiation under physiological conditions. Cardiovasc Res. 2014;103:554–563. doi: 10.1093/cvr/cvu158

26. Eisner D, Bode E, Venetucci L, Trafford A. Calcium flux balance in the heart. J Mol Cell Cardiol. 2013;58:110–117. doi: 10.1016/j.yycj.2012.11.017

27. Díaz ME, Trafford AW, Eisner DA. The effects of exogenous calcium buffers on the systolic calcium transient in rat ventricular myocytes. Biophys J. 2001;80:1915–20. doi: 10.1016/S0006-3495(01)07616-9

28. Steenbergen C, Murphy E, Levy L, London RE. Elevation in cytosolic free Ca\(^{2+}\) and underlies disturbed calcium handling in the rabbit rabbit pressure and volume overload heart failure model. Cardiovasc Res. 2003;57:1015–1024. doi: 10.1016/s0008-6366(02)00809-x

29. Beuckelmann DJ, Nääbauer M, Erdmann E. Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. Circulation. 1992;85:1008–1015. doi: 10.1161/01.atri.85.3.1014

30. Wier WG, Hess P. Excitation-contraction coupling in cardiac Purkinje fibres. Effects of cardiotoxic steroids on the intracellular [Ca\(^{2+}\)] transient, membrane potential, and contraction. J Gen Physiol. 1984;83:395–415. doi: 10.1085/jgp.83.3.395

31. Eisner DA, Lederer WJ, Vaughan-Jones RD. The quantitative relationship between twitch tension and intracellular sodium activity in sheep Purkinje fibres. J Physiol. 1984;355:251–266. doi: 10.1113/jphysiol.1984.sp015417

32. Eisner DA, Lederer WJ. Inotropic and arrhythmogenic effects of potassium-depleted solutions on mammalian cardiac muscle. J Physiol. 1997;501:1109–1125. doi: 10.1113/jphysiol.1997.sp032080

33. Harrison SM, McCall E, Boyett MR. The relationship between contraction and intracellular sodium in rat and guinea-pig ventricular myocytes. J Physiol. 1992;449:517–530. doi: 10.1113/jphysiol.1992.sp019100

34. Stafford N, Wilson C, Oceandy D, Neyes L, Carwright EJ. The plasma membrane calcium ATPases and their role as major new players in human disease. Physiol Rev. 2017;97:1089–1125. doi: 10.1152/physrev.00028.2016

35. Mohamed TM, Oceandy D, Zi M, Prehar S, Alatiw N, Wang Y, Shaheen MA, Abou-Leisa R, Schelcher C, Hegab Z, et al. Plasma membrane calcium pump (PMCA4)-neuronal nitric-oxide synthase complex regulates cardiac contractility through modulation of a compartmentalized cyclic nucleotide microdomain. J Biol Chem. 2011;286:41520–41529. doi: 10.1074/jbc.M111.290411

36. Bassani R, Bassani JW, Bers DM. Mitochondrial and sarcocellum Ca\(^{2+}\) transport reduce [Ca\(^{2+}\)] during caffeine contractions in rabbit cardiac myocytes. J Physiol. 1992;445:591–608. doi: 10.1113/jphysiol.1992.sp019249

37. Varo A, Negretti N, Hester SB, Eisner DA. An estimate of the calcium content of the sarcoplasmic reticulum in rat ventricular myocytes. Pflugers Arch. 1993;423:158–160. doi: 10.1007/bf03749795

38. O’Neill SC, Vádeolmillos M, Lamont C, Donoso P, Eisner DA. The contribution of Na-Ca exchange to relaxation in mammalian cardiac muscle. Ann N Y Acad Sci. 1991;639:444–452. doi: 10.1111/j.1749-6632.1991.tb17331.x
91. Layland, J. Kentish JC. Positive force- and [Ca\(^{2+}\)]-frequency relationships in rat ventricular trabeculae at physiological frequencies. *Am J Physiol.* 1999;276:H9–H18. doi: 10.1152/ajpheart.1999.276.1.H9

92. Antoons G, Mubagwa K, Nevelsteen I, Spido KR. Mechanisms underlying the frequency dependence of contraction and [Ca\(^{2+}\)] transfer in mouse ventricular myocytes. *J Physiol.* 2002;543:889–898. doi: 10.1113/jphysiol.2002.052619

93. Frampton JE, Orchard CH, Boyett MR. Diastolic, systolic and sarcoplasmic reticulum [Ca\(^{2+}\)] during inotropic interventions in isolated rat myocytes. *J Physiol.* 1991;437:381–375. doi: 10.1113/jphysiol.1991.sp018600

94. Dibb KM, Eisner DA, Trafford AW. Regulation of systolic [Ca\(^{2+}\)]i and cardiac cytoskeletal structure during inotropic interventions in isolated rat ventricular trabeculae at physiological frequencies. *J Physiol.* 2007;585:579–592. doi: 10.1113/jphysiol.2007.141473

95. Honvíd G, Szendrász G, Veress R, Almássy J, Magyar J, Bányász T, Tóth A, Papp Z, Nánási PP. Frequency-dependent effects of omeprazole on cell shortening of isolated canine ventricular cardiomyocytes. *Naunyn Schmiedebergs Arch Pharmacol.* 2017;390:1239–1246. doi: 10.1007/s00210-017-1422-z

96. Pieske B, Kretschmann B, Meyer M, Holubarsch C, Weirich J, Posival H, Bowey S, Travers AM, Stienen GJ, Steele DS, White E. Decreased creatine kinase is linked to diastolic stiffness in heart failure patients with mid-range and preserved ejection fraction. *Circulation Heart Fail.* 2018;11:1559–1566. doi: 10.1161/CIRCHEARTFAILURE.117.0062087

97. Bell SP, Nyland L, Tischler MD, McNabb M, Granzier H, LeWinter MM. [Ca\(^{2+}\)]-dependent effects of ADP and Ca\(^{2+}\) on contractile and diastolic function in rat papillary muscle. *J Biol Chem.* 2005;280:5651–5658. doi: 10.1074/jbc.M503510200

98. Frampton JE, Harrison SM, Boyett MR, Orchard CH. Ca\(^{2+}\) and Na\(^{+}\) in rat ventricular myocytes showing different force-frequency relationships. *Am J Physiol. Cell Physiol.* 1991;261:C739–C750. doi: 10.1152/ajpcell.1991.261.5.C739

99. Mullen LA, Hasenfuss G, Leavitt B, Allen PD, Alpert NR. Altered myocardial force-frequency relation in human dilated cardiomyopathy. *Circulation.* 1995;92:1169–1176. doi: 10.1161/01.01.95.1169

100. Bountis C, Kaila K, Vaughan-Jones RD. Effect of repetitive activity upon intracellular pH, sodium and contraction in sheep cardiac Purkinje fibres. *J Physiol.* 1988;398:341–360. doi: 10.1113/jphysiol.1988.sp010746

101. Cohen CJ, Fozzard HA, Sheu SS. Increase in intracellular sodium ion activity during stimulation in mammalian cardiac muscle. *Circ Res.* 1992;65:651–662. doi: 10.1161/01.01.65.651

102. Yellin EL, Nikolic S, Frater RW. Left ventricular filling dynamics and diastolic function. *Prog Cardiovasc Dis.* 1990;33:247–271. doi: 10.1016/0033-0620(90)90016-6

103. Cheng CP, Ignacio T, Littlic W. Mechanism of augmented rate of left ventricular filling during exercise. *Circ Res.* 1992;70:9–19. doi: 10.1161/01.01.70.9

104. Borlaug BA, Jaber WA, Ommen SR, Lam CS, Redfield MM, Nishimura RA. Altered relaxation and compliance reserve during dynamic exercise in heart failure with preserved ejection fraction. *Heart.* 2011;97:964–969. doi: 10.1136/hrt.2010.212797

105. Bortolotti E, Citron J. Myocardial cardiovascular regulation and its contribution to cardiac function after beta-adrenergic stimulation in cardiac myocytes. *Cardiovasc Res.* 2014;104:347–354. doi: 10.1093/ctx/cvu021

106. Yellin EL, Nikolaic S, Frater RW. Left ventricular filling dynamics and diastolic function. *Prog Cardiovasc Dis.* 1990;33:247–271. doi: 10.1016/0033-0620(90)90016-6

107. Van Heerebeek L, Borbély A, Niessen HW, Bronzwaer JG, von der Velden J, Stienen GJ, Linke WA, Laarman GJ, Paulus WJ. Myocardial structure and function differ in systolic and diastolic heart failure. *Circulation.* 2006;113:1966–1973. doi: 10.1161/CIRCULATIONAHA.105.585719

108. Fowler ED, Benoist M, Drinkhill MJ, Stienen GJ, White E, Steele DS, Porrello ER, Erickson JR, Dibb KM, Boyett MR. Cardiac structure and function in heart failure with preserved ejection fraction: a randomized clinical trial. *JAMA.* 2013;309:1268–1277. doi: 10.1001/jama.2013.20309

109. Kimura M, Komatsu T, Tani T, Kato S, Saito N, Kirigaya H, Gyotoku D, Iinuma N, Kusanakwa Y, Ighuchi K, Nakachi T, Fukui K, Futaki M, et al. Prognostic significance of quantitative assessment of focal myocardial fibrosis in patients with heart failure with preserved ejection fraction. *Int J Cardiol.* 2015;191:314–319. doi: 10.1016/j.ijcard.2015.05.048

110. Pfeffer MA, Shah AM, Borlaug BA. Heart failure with preserved ejection fraction: exercise echocardiography reveals complex abnormalities of both systolic and diastolic ventricular function involving torsion, untwist, and longitudinal motion. *J Am Coll Cardiol.* 2009;54:36–46. doi: 10.1016/j.jacc.2009.03.037

111. Tóth A, Papp Z, Nánási PP. Frequency-dependent effects of omecamtiv mecarbil on cell shortening of isolated canine ventricular cardiomyocytes. *Naunyn Schmiedebergs Arch Pharmacol.* 2017;390:1239–1246. doi: 10.1007/s00210-017-1422-z

112. Yellin EL, Nikolic S, Frater RW. Left ventricular filling dynamics and diastolic function. *Prog Cardiovasc Dis.* 1990;33:247–271. doi: 10.1016/0033-0620(90)90016-6
myocardial passive stiffness by phosphorylation of the thin filaments. Circ Res. 2009;105:631–638, 617 p following 638. doi: 10.1161/CIRCRESAHA.109.198465

130. Hamdani N, Krysiaj K, Kreuzler MM, Neef S, Dos Remedios CG, Maier LS, Krüger M, Backs J, Linke WA. Crucial role for Ca2+-/calmodulin-dependent protein kinase-II in regulating diastolic stress of normal and failing hearts via titin phosphorylation. Circ Res. 2013;112:664–674. doi: 10.1161/CIRCRESAHA.113.300105

131. Grützner A, García-Manyes S, Kötter S, Badilla CL, Fernandez JM, Linke WA. Modulation of titin-based stiffness by disulfide bonding in the cardiac titin N2-B unique sequence. Biophys J. 2009;97:825–834. doi: 10.1016/j.bpj.2009.05.037

132. Alegre-Cebollada J, Kosiú P, Eckels E, Rivas-Pardo JA, Hamdani N, Warren CM, Solaro RJ, Linke WA, Fernández JM. S-glutathionylation of cryptic cysteines enhances titin elasticity by blocking protein folding. Cell. 2014;156:1235–1246. doi: 10.1016/j.cell.2014.01.056

133. Labeit D, Wałatanka K, Witz C, Fujita H, Wu Y, Lahmers S, Funck T, Labeit D, Fujita H, Labeit D, Gerull B, Labeit S, Granzier HL. Titin isoform-dependent effect of calcium on passive myocardial tension. Am J Physiol Heart Circ Physiol. 2004;287:H2528–H2534. doi: 10.1152/ajpheart.00553.2004

134. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

135. Okuda K, Furukawa W, Nakamura H, Tani H, Ogawa Y, Eto T, Georgiou I. Titin disulide bond and phosphorylation regulates cardiac diastolic relaxation and fine-tunes the Frank-Starling response. Nat Commun. 2016;7:13187. doi: 10.1038/ncomms13187

136. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

137. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

138. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

139. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

140. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

141. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

142. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

143. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

144. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

145. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

146. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

147. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

148. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

149. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

150. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

151. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

152. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

153. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

154. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

155. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380

156. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–1273. doi: 10.1161/CIRCRESAHA.107.156380
164. Hamouda NN, Sydorenko V, Quareshi MA, Alkaabi JM, Oz M, Howarth FC. Dapagliflozin reduces the amplitude of shortening and Ca\textsuperscript{2+} transient in ventricular myocytes from streptozotocin-induced diabetic rats. Mol Cell Biochem. 2015;400:57–68. doi: 10.1007/s11010-014-2262-5

165. Noda N, Hayashi H, Miyata H, Suzuki S, Kobayashi A, Yamazaki N. Cytosolic Ca\textsuperscript{2+} concentration and pH of diabetic rat myocytes during metabolic inhibition. J Mol Cell Cardiol. 1992;24:435–446. doi: 10.1016/0022-2828(92)90293-2

166. Zhang L, Ward ML, Phillips AR, Zhang S, Kennedy J, Barry B, Cannell MB, Cooper GJ. Protection of the heart by treatment with a divalent-copper-selective chelator reveals a novel mechanism underlying cardiomyopathy in diabetic rats. Cardiovasc Diabetol. 2013;12:123. doi: 10.1186/1475-2840-12-123

167. Miranda-Silva D, Wust RC, Conceicao G, Goncalves-Rodrigues P, Goncalves N, Goncalves A, Kuster DW, Leite-Moreira AF, van der Velden J, de Sousa Beleza JM, et al. Disturbed cardiac mitochondrial and cytosolic calcium handling in a metabolic-risk related rat model of heart failure [published online September 13, 2019]. Acta Physiol (Oxf). 2019:e13378. doi: 10.1111/apha.13378. https://onlinelibrary.wiley.com/doi/full/10.1111/apha.13378

168. Hohendanner F, Bode D, Primessnig U, Guthof T, Doerr R, Jeuthe S, Reimers S, Zhang K, Bach D, Wakula P, et al. Cellular mechanisms of metabolic syndrome-related atrial decompensation in a rat model of HFpEF. J Mol Cell Cardiol. 2018;115:10–19. doi: 10.1016/j.yjmcc.2017.12.012

169. Schiattarella GG, Altamirano F, Tong D, French KM, Villalobos E, Kim SY, Luo X, Jiang N, May HI, Wang ZV, et al. Nitrosative stress drives heart failure with preserved ejection fraction. Nature. 2019;568:351–356. doi: 10.1038/s41586-019-1100-z

170. Selby DE, Palmer BM, LeWinter MM, Meyer M. Tachycardia-induced diastolic dysfunction and resting tone in myocardium from patients with a normal ejection fraction. J Am Coll Cardiol. 2011;58:147–154. doi: 10.1016/j.jacc.2010.10.069

171. Frey N, Luedde M, Katus HA. Mechanisms of disease: hypertrophic cardiomyopathy. Nat Rev Cardiol. 2011;9:91–100. doi: 10.1038/nrcardio.2011.159

172. Despa S, Islam MA, Weber CR, Pogwizd SM, Bers DM. Intracellular Na\textsuperscript{+} concentration is elevated in heart failure but Na/P pump function is unchanged. Circulation. 2002;105:2543–2548. doi: 10.1161/01.cir.0000069686.31472.C5

173. Hasenfuss G, Schillinger W, Lehnart SE, Preuss M, Pieske B, Maier LS, Prestle J, Minami K, Just H. Relationship between Na\textsuperscript{+}-Ca\textsuperscript{2+}-exchanger protein levels and diastolic function of failing human myocardium. Circulation. 1999;99:641–648. doi: 10.1161/01.cir.99.5.641

174. Sossalla S, Wagner S, Rasenack EC, Ruff H, Weber SL, Schöndube FA, Tirilomis T, Tenderich G, Hasenfuss G, Belardinelli L, et al. Ranolazine improves diastolic dysfunction in isolated myocardium from failing human hearts—role of late sodium current and intracellular ion accumulation. J Mol Cell Cardiol. 2008;45:32–43. doi: 10.1016/j.yjmcc.2008.03.006

175. Cappetta D, Esposito G, Coppini R, Piegari E, Russo R, Cuiffreda LP, Rivellino A, Santini L, Rafaniello C, Scavone C, et al. Effects of ranolazine in a model of doxorubicin-induced left ventricle diastolic dysfunction. Br J Pharmacol. 2017;174:3696–3712. doi: 10.1111/bph.13791

176. Undrovinas NA, Maltsev VA, Belardinelli L, Sabbah HN, Undrovinas A. Late sodium current contributes to diastolic cell Ca\textsuperscript{2+} accumulation in chronic heart failure. J Physiol Sci. 2010;60:245–257. doi: 10.1007/s12576-010-0092-0

177. Zhang T, Maier LS, Dalton ND, Miyamoto S, Ross J Jr, Bers DM, Brown JH. The deltaC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. Circ Res. 2003;92:912–919. doi: 10.1161/01.RES.0000069686.31472.C5

178. Sossalla S, Maurer U, Schotola H, Hartmann N, Didié M, Zimmermann WH, Jacobshagen C, Wagner S, Maier LS. Diastolic dysfunction and arrhythmias caused by overexpression of CaMKII\textsubscript{δ} can be reversed by inhibition of late Na\textsuperscript{+} current. Basic Res Cardiol. 2011;106:263–272. doi: 10.1007/s00395-010-0136-x

179. Runte KE, Bell SP, Selby DE, Haussler TN, Ashikaga T, LeWinter MM, Palmer BM, Meyer M. Relaxation and the role of calcium in isolated contracting myocardium from patients with hypertensive heart disease and heart failure with preserved ejection fraction. Circ Heart Fail. 2017;10:e004311.