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# CaMKII: A link between metabolic disorders and cardiac arrhythmias

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ABSTRACT

The prevalence of metabolic diseases -such as obesity, prediabetes, metabolic syndrome or diabetes-has increased globally over the years. These diseases, mainly diabetes mellitus (DM), are among the leading causes of mortality and morbidity worldwide, with increased risk of diabetic cardiomyopathy (DC), cardiac arrhythmias, and heart failure (HF).

In metabolic diseases several steps and proteins involved in excitation-contraction coupling (ECC) are compromised, precluding an efficient and rhythmic cardiac contraction; moreover, calcium/calmodulin-dependent protein kinase II (CaMKII) a kinase involved in ECC, is upregulated in several metabolic maladies and significantly contributes to cardiac remodeling and arrhythmias, among which are calcium ($Ca^{2+}$)-triggered arrhythmias. CaMKII activation is canonically produced by an increase and binding of $Ca^{2+}$-calmodulin followed by auto-phosphorylation; furthermore, it may occur as a result of several post-translational modifications, including oxidation and O-GlcNAcylation that are usually present in metabolic illnesses and support chronic CaMKII upregulation and ECC impairment.

The aim of the present review is to summarize what is known about the different pathways modifying CaMKII activity and $Ca^{2+}$ handling in metabolic disorders and may promote $Ca^{2+}$-triggered arrhythmias even at the early stages of metabolic alterations. Future challenges in this field may encompass unraveling the precise mechanisms by which metabolic disturbances modulate CaMKII activity and its downstream effects on cardiac electrophysiology among different species. This would allow the development of robust and valid preclinical human models that can accurately recapitulate the pathophysiology of the human heart. Additionally, investigating the impact of targeted interventions, such as pharmacological inhibitors or gene therapies, could provide valuable insights into the feasibility and efficacy of manipulating CaMKII signaling to prevent or treat arrhythmias in metabolic disorders.

## 1. Introduction

Metabolic diseases are a group of illnesses that disrupt normal metabolism. They include obesity, metabolic syndrome (MS), prediabetes, and diabetes mellitus (DM).

Obesity estimates suggest that over one-third of the world population is overweight or obese (World Health Organization, 2022). Obesity is closely associated with MS, a constellation of comorbid conditions, including impaired fasting glucose, insulin resistance, hypertension, dyslipidemias, and central obesity. The Joint Scientific Declaration, published in 2009, characterizes MS as a complex of risk factors related to DM and the cardiovascular system (Saeedi et al., 2019). Indeed, all these conditions have been linked to adverse cardiovascular prognoses with an increased risk of arrhythmias and mortality (See below and (Grisanti, 2018; Narayanan et al., 2015)).

DM is a chronic metabolic disorder commonly characterized by high blood glucose levels. It results from defects in insulin production, insulin resistance, or both. DM affects millions of people worldwide and its prevalence is increasing exponentially (Cho et al., 2018). It has been early recognized that one of the more important complications of DM resides at the heart level, with the development of diabetic cardiomyopathy (DC) (Kannel et al., 1974).

DC usually initiates with diastolic dysfunction, which is HF with preserved ejection fraction, HFpEF (EF $\geq$ 50%). Later systolic dysfunction develops which eventually leads to systolic HF with EF<40% (Ponikowski et al., 2016). The underlying mechanisms of these

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alterations are complex; however, it is known that impaired $Ca^{2+}$ handling is one of the main players in the electrical and mechanical dysregulations that culminate in ventricular arrhythmias and HF in this disease (Shao et al., 2007a; Stølen et al., 2009).

Arrhythmias may originate through different mechanisms like repolarizations or conduction defects, systemic factors, or alterations in $Ca^{2+}$ handling, which leads to $Ca^{2+}$-triggered arrhythmias. This holds true for several cardiac pathologies of various origins (Chelu et al., 2009; Luo et al., 2013; Pogwizd and Bers, 2004; Said et al., 2008, 2011), among which is DC (Stølen et al., 2009; Shao et al., 2007b) (Fig. 1). In this context, CaMKII is a serine/threonine kinase that mediates physiological responses upon acute β-adrenergic activation (Ai et al., 2005; Lindemann and Watanabe, 1985; Mundiña-Weilenmann et al., 1996). However, it also plays a pathological role under situations of cardiac stress, including DC. In DC CaMKII is upregulated and significantly contributes to detrimental effects of the disease (See below).

In this review, we will first give a brief overview of $Ca^{2+}$ handling in cardiomyocytes and the main characteristics of CaMKII. We will then discuss the mechanisms of $Ca^{2+}$-triggered cardiac arrhythmias in metabolic disorders, with a special focus on CaMKII acting on cardiac myocytes. We will also briefly refer to the possible contribution to triggered arrhythmias of non-myocyte cardiac cells, which are also affected in these diseases. The understanding of CaMKII signaling and arrhythmia mechanisms may reveal new therapeutic targets and ultimately better treatment in diabetic cardiomyopathy and related metabolic diseases.

### 1.1. *Excitation-contraction coupling (ECC) and the regulation of sarcoplasmic reticulum -$Ca^{2+}$ handling proteins*

In each cardiac cycle, depolarization of the cardiomyocyte membrane allows $Ca^{2+}$ entry through the L-type $Ca^{2+}$ channels (LTCCs). $Ca^{2+}$ ions bind to the ryanodine receptors (RyR2s) inducing a larger release of $Ca^{2+}$ from the sarcoplasmic reticulum (SR) that evokes contraction of the myofilaments. $Ca^{2+}$ is then re-uptaken by the $Ca^{2+}$-ATPase of the SR (SERCA2a), and a small amount is extruded from the cell through the sodium ($Na^{+}$)-$Ca^{2+}$ exchange mechanism (NCX) and the plasma membrane $Ca^{2+}$ pump (PMCA), leading to relaxation (Fig. 2). Approximately 1% of the systolic $Ca^{2+}$ is re-uptaken by the mitochondria (Bers, 2002).

*Worth mentioning, 1*. Under conditions of intracellular $Ca^{2+}$ balance, the extrusion mechanisms draw out of the cell the same amount of $Ca^{2+}$ that enters through the LTCCs; *2.* SR $Ca^{2+}$ uptake is a key process in the relaxation of cardiomyocytes and in the proper storage of SR $Ca^{2+}$ content for the next contraction. For instance, a decrease in SR $Ca^{2+}$ uptake in HF due to a decrease in the expression and activity of SERCA2a, is a main responsible for the diastolic and systolic deficiencies, typical of this illness (de la Bastie et al., 1990); *3.* The NCX is electrogenic, i.e., it pumps one $Ca^{2+}$ out in exchange for the influx of three $Na^{+}$ ions into the cell. As will be discussed below, exacerbation of $Ca^{2+}$ efflux through the NCX, by increasing $Na^{+}$ influx and depolarizing cell membrane, may favor ectopic beats and $Ca^{2+}$-triggered arrhythmias.

Both SERCA2a and RyR2 are under the tight control of different proteins. Phospholamban (PLN) is the main and first discovered SERCA2a regulator (Tada et al., 1975). PLN inhibits SERCA2a tonically and reversibly, and *in vivo* PLN phosphorylation, either by PKA, at $Ser^{16}$ site, or CaMKII at $Thr^{17}$ site, relieves this inhibition, increasing SERCA2a activity, SR $Ca^{2+}$ reuptake and cardiomyocytes relaxation. In turn, the increase in SR $Ca^{2+}$ reuptake, increases SR $Ca^{2+}$ load and SR $Ca^{2+}$ release, enhancing cardiomyocytes contractility (Mundiña-Weilenmann et al., 1996; Luo et al., 1994). After the discovery of PLN, several other regulatory proteins have emerged, which interact with and/or produce post-translational modifications of the SERCA2a-PLN duet, revealing a fine and tight tuning of the reuptake process (*See for review* (Federico et al., 2020; Kranias and Hajjar, 2012)).

RyR2s are also highly controlled by proteins assembled in a macromolecular complex that includes several regulatory molecules, such as calmodulin (CaM), calsequestrin-2 (CASQ2), Junctin, Triadin, and FK-506 binding proteins (FKBP12/12.6). Other proteins in the RyR2 complex regulate the level of RyR2 phosphorylation, like protein phosphatases and kinases, including CaMKII. Indeed, RyR2 are phosphorylated by PKA, at $Ser^{2808/09}$ and $Ser^{2030/31}$ residues, by CaMKII, at $Ser^{2808/09}$ and more importantly at $Ser^{2814/15}$ site, and by PKG at $Ser^{2808/09}$ site. Whereas there are numerous evidences supporting that phosphorylation of $Ser^{2814}$ by CaMKII enhances RyR2 activity (Ai et al., 2005), the results remain controversial about the role of PKA-dependent phosphorylation at $Ser^{2808}$ and $Ser^{2030}$ under both, physiological and pathological conditions (Carter et al., 2006; Kushnir et al., 2018; Marx et al., 2000; Wehrens et al., 2006; Wei et al., 2021; Xiao et al., 2006). In the last few years, a new kinase regulator of RyR2 emerged, the striated muscle preferentially expressed protein (SPEG). Unlike phosphorylation by other kinases, SPEG phosphorylation of RyR2 at $Ser^{2367}$ site reduces RyR2 activity (Campbell et al., 2020; Quick et al., 2017). The RyR2s are also regulated by S-nitrosylation and oxidation (Marengo et al., 1998; Sun et al., 2008) (See for review (Federico et al., 2020)). Interestingly, some of these regulatory proteins, like histidine-rich $Ca^{2+}$ binding protein (HRC) (Arvanitis et al., 2018), c-Jun N-terminal kinase isoform 2 (JUNK2) (Yan et al., 2021), and SPEG, interact with both, SERCA2a and RyR2.

The high regulatory control of each step of ECC allows for a fine-tuning of myocyte $Ca^{2+}$ handling, revealing the importance of maintaining intracellular $Ca^{2+}$ homeostasis for cardiac function. Any disruption of this delicate balance may compromise the precise coordination required for optimal cardiac performance. As will be discussed below, it is known that some of these regulations are broken in several cardiomyopathies, including DC, playing a significant role in $Ca^{2+}$-triggered arrhythmias.

### 1.2. *$Ca^{2+}$-calmodulin-dependent protein kinase (CaMKII)*

The reversible phosphorylation of proteins is one of the most important mechanisms in the regulation of cellular function. Virtually every biochemical process in eukaryotic cells is under the control of post-translational modifications via phosphorylation of key regulatory proteins, which in turn trigger the functional response to a variety of stimuli.

Although c-AMP–dependent processes have hystorically attracted the most attention as modulators of cardiac protein phosphorylation, several other kinases emerged with strong physiological and pathological roles, among which is CaMKII.

CaMKII is a multimeric holoenzyme composed of 6–12 subunits that exists in four isoforms encoded by four separate conserved genes; α, β, γ, and δ (Braun and Schulman, 1995). Whereas the α and β isoforms are expressed predominantly in neuronal tissue, the δ isoform of the enzyme predominates in the heart. Two major splice variants of CaMKIIδ are expressed in the adult heart, $CaMKII\delta_B$ and $CaMKII\delta_C$. The former, which contains an 11 amino acid nuclear localization sequence, is therefore predominantly, although not exclusively, located at the nucleus (Mishra et al., 2011).

Each of the dozen CaMKII monomers that compose the holoenzyme consists of three distinct domains: the association domain which directs holoenzyme assembly, the regulatory domain, which controls activation of the enzyme, and the catalytic domain which associates with substrates and performs the kinase function. CaMKII indirectly senses the increase in intracellular $Ca^{2+}$ by binding of $Ca^{2+}$–CaM at the CaM-binding region in the regulatory domain. The binding of the $Ca^{2+}$–CaM complex disrupts autoinhibitory interactions, allowing substrates and ATP to gain access to the catalytic domain. Simultaneous $Ca^{2+}$–CaM binding to adjacent subunits within the same holoenzyme, results in the rapid autophosphorylation of $Thr^{287}$ or $Thr^{286}$ site, according to the isoform. Autophosphorylation of CaMKII increases the affinity of the kinase to $Ca^{2+}$ and CaM and results in autonomous activity, independent of intracellular $Ca^{2+}$ ($Ca_i^{2+}$). Therefore, transient

elevations of $Ca_i^{2+}$ can generate a prolonged response through the constitutive activity of autophosphorylated CaMKII, which confers the unique property of "memory" to this enzyme. CaMKII autonomous activity is also produced by conditions favoring oxidation of a pair of methionine residues ($Met^{281/282}$), nitrosylation of $Cys^{272/290}$ sites and glycosylation of $Ser^{280}$ residue (Erickson et al., 2008, 2013; Hegyi et al., 2021a) (Fig. 3). Interestingly, the close disposition of the post-translational modification sites at the regulatory CaMKII domain may facilitate their interaction. Moreover, it is shown that increased oxidation shifts the $Ca^{2+}$ dependence for CaMKII activation to extremely low levels of $Ca^{2+}$, which would favor CaMKII activation even under basal or subdiastolic intracellular $Ca^{2+}$ levels (Palomeque et al., 2009).

Phosphorylation of CaMKII is also greatly dependent on the activity of CaMKII-specific phosphatases. Four species of protein phosphatases, PP1, PP2A, PP2C and a novel family of specific CaMKII phosphatases, have been reported to dephosphorylate and negatively regulate CaMKII (Ishida et al., 2003). Among the phosphorylation targets of CaMKII there are several $Ca^{2+}$ handling proteins, such as the LTCC (Anderson et al., 1994), the RyR2 (Ferrero et al., 2007; Wehrens et al., 2004), and PLN (Mundiña-Weilenmann et al., 1996).

It has been shown that CaMKII plays a physiological or beneficial role, such as during β-adrenoceptor stimulation (Lindemann and Watanabe, 1985; Mundiña-Weilenmann et al., 1996), pH regulation (Vila--Petroff et al., 2010) or the recovery of contractility during stunning and acidosis (Mundiña-Weilenmann et al., 2005; Said et al., 2003; Valverde et al., 2006). However, a review of the literature indicated that enhanced CaMKII activity is mainly associated with detrimental effects such as cardiac inflammation (Rusciano et al., 2019; Singh and Anderson, 2011), hypertrophy (Anderson et al., 2011; Backs et al., 2009; Velez Rueda et al., 2012) ischemia/reperfusion injury (Di Carlo et al., 2014; Salas et al., 2010; Vila-Petroff et al., 2007), HF (Ai et al., 2005; Bossuyt et al., 2008) and $Ca^{2+}$-triggered arrhythmias under different pathological conditions that include DM, prediabetes and obesity (Chelu et al., 2009; Said et al., 2008, 2011; Shao et al., 2007b; Ai et al., 2005; Erickson et al., 2013; Hegyi et al., 2021a; Gonano et al., 2011; Lascano et al., 2013; Mazzocchi et al., 2016; Santalla et al., 2014; Sepúlveda et al., 2017; Sommese et al., 2016; Tse et al., 2016; Valverde et al., 2019; Joseph et al., 2019; Chan et al., 2019). In summary, CaMKII activity is enhanced by increasing intracellular $Ca^{2+}$, but also by oxidation, nitrosylation, and glycosylation. These multiple stimuli which are usually activated under pathological conditions, may explain why the negative effects of this kinase often overshadow its physiological or beneficial actions.

### 1.3. $Ca^{2+}$-triggered arrhythmias

Altered ionic currents and enhanced SR $Ca^{2+}$ leak increase the susceptibility for early and delayed afterdepolarizations (EADs, DADs) as well as spontaneous action potentials (AP), representing the arrhythmogenic trigger. EADs are defined as a slowing or reversal of normal repolarization that occurs before the completion of an AP, usually during phase 2 or 3 of human AP, whereas DADs occur after AP completion (Fig. 4A).

#### 1.3.1. Early afterdepolarizations

EADs occurs usually in the setting of prolonged repolarization and are classically attributed to the reactivation of $Ca^{2+}$ current (ICa) (Nuss et al., 1999). A second major current that facilitates EADs formation is NCX. The late component of the $Na^+$ current (INaL) has been recognized as an important player to set up the conditions for EADs, by producing SR $Ca^{2+}$ overload, via the reduction of repolarization reserve and the increase in intracellular $Na^+$ concentration. CaMKII significantly enhances INaL, which represents a tiny fraction of $Na^+$ channels that may remain open/reopen to produce a non-inactivating or persistent $Na^+$ current under a sustained depolarization such as the plateau phase of AP in ventricular cardiomyocytes (Sato et al., 2017).

#### 1.3.2. Delay afterdepolarizations

SR $Ca^{2+}$ release in the intact cardiomyocytes occurs via local events referred to as $Ca^{2+}$ sparks (Cheng et al., 1993),. These events are visualized as small, localized increases in cytosolic $Ca^{2+}$ and they occur at a very low frequency during rest, in a stochastic manner, even in the absence of cell membrane depolarization and $Ca^{2+}$ influx (Fig. 4B). However, when the SR $Ca^{2+}$ load exceeds a threshold, which is largely determined by the state of the RyR2, an increase in SR $Ca^{2+}$ sparks ($Ca^{2+}$ leak) may occur. These abnormal spontaneous $Ca^{2+}$ discharges from the SR may propagate as regenerative $Ca^{2+}$ waves through cardiac cells (Laurita and Rosenbaum, 2008) (Fig. 4A and C). Spontaneous $Ca^{2+}$ waves are arrhythmogenic because they activate inward membrane currents, mainly through the electrogenic NCX working in the forward mode. $Na^+$ influx through the NCX depolarizes the cell membrane (DAD). If this depolarization reaches the excitability threshold, an ectopic beat (an extrasystole) may occur (Luo et al., 2013; Pogwizd and Bers, 2004; Laurita and Rosenbaum, 2008) (Fig. 4A).

At the cell level, one limitation for arrhythmias occurrence is that SR $Ca^{2+}$ leak can only persist if there is a simultaneous increase in SR $Ca^{2+}$ uptake. This is because the SR must maintain a certain $Ca^{2+}$ level (SR $Ca^{2+}$ threshold) to support $Ca^{2+}$ sparks. As previously shown by Eisner's group (Trafford et al., 2000), increasing the $P_0$ of RyR2 by the application of low caffeine concentrations results in an increase in $Ca^{2+}$ transient amplitude due to the increase in RyR2 $P_0$. However, the increase in RyR2 $P_0$ also increases SR $Ca^{2+}$ leak, decreasing SR $Ca^{2+}$ content and suppressing both, the increase in cytosolic $Ca^{2+}$ transient and the enhanced diastolic $Ca^{2+}$ leak, despite the increase in RyR2 $P_0$ was still present. Similarly, isolated cardiomyocytes in which $Ser^{2814}$ site of RyR2 was constitutively pseudophosphorylated to produce a "leaky" SR (S2814D mice), did not show a higher frequency of $Ca^{2+}$ sparks with respect to wild type (WT) cardiomyocytes consistent with the reduced SR $Ca^{2+}$ content of S2814D cardiomyocytes (Mazzocchi et al., 2016; Valverde et al., 2019). Therefore, an increase in SR $Ca^{2+}$ leak may only be arrhythmogenic when it is associated with an increase in SR $Ca^{2+}$ uptake, able to maintain SR $Ca^{2+}$ load (Fig. 4D). Several studies described an increase in SR $Ca^{2+}$ uptake in DC and prediabetic heart, supporting the persistent nature of SR $Ca^{2+}$ leak in this cardiomyopathy (See below). However, even if there is no associated permanent increase in SR $Ca^{2+}$ uptake, the arrhythmic propensity of these myocytes would appear when they are submitted to stress conditions (Chelu et al., 2009; Gonano et al., 2011; Mazzocchi et al., 2016; Sommese et al., 2016; Van Oort et al., 2010) (Right panel of Fig. 4D).

At the tissue level, a second important limitation for an extrasystole to occur is the number of neighbor cells experimenting DADs at the same time, i.e. the number of cells losing their capacity to act as an electrotonic current provider for minimizing the voltage difference. Using a rabbit ventricular AP cell model, Xie et al. (2010), estimated that the number of cardiomyocytes required to reach the AP threshold exceeds 800 thousand susceptible cells. This number decreases dramatically in the presence of fibrosis, gap junction uncoupling, HF-like remodeling, post-infarction scars, among others, favoring triggered activity (Xie et al., 2010). Although it will not be discussed in this review, these conditions are present in DC; for instance in diabetic hearts, there is a reduction of Connexin 43 (Cx43) expression, Cx43 subcellular redistribution and an increased fibrosis that significantly decreases cell-to-cell coupling, reducing conduction velocity and favoring arrhythmias occurrence (Pereira et al., 2014; Watanabe et al., 2012).

In summary, the occurrence of $Ca^{2+}$-triggered arrhythmias holds significant importance in the development and progression of cardiac arrhythmias. Aberrant $Ca^{2+}$ handling disrupts the normal rhythm of the heart, leading to disturbances in cardiac conduction, increased risk of reentry circuits, and the potential for life-threatening arrhythmias.

### 1.4. CaMKII and $Ca^{2+}$ triggered arrhythmias in diabetic cardiomyopathy

As already discussed, and shown in Fig. 1, different mechanisms may

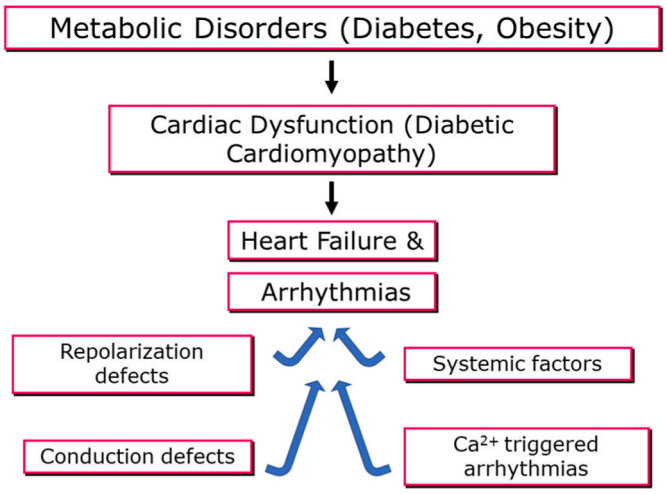


**Fig. 1.** Arrhythmias in metabolic disorders.
Metabolic disorders like diabetes or obesity may lead to cardiac dysfunction that eventually culminates in heart failure and ventricular arrhythmias. Ventricular arrhythmias in diabetic cardiomyopathy may have multiple and even superimposed origins.

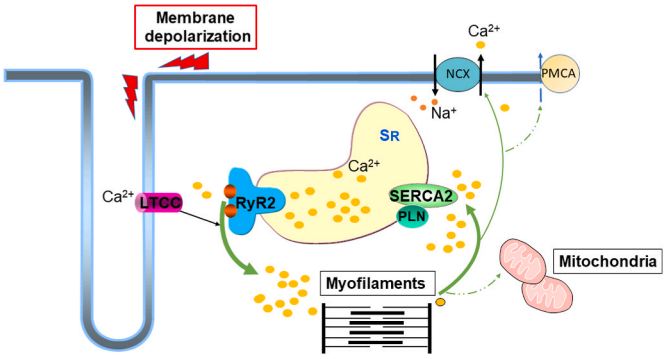


**Fig. 2.** Scheme of the excitation-contraction coupling in cardiac myocytes.
**LTTC:** L-type $Ca^{2+}$ channels, **SR**: Sarcoplasmic reticulum, **RyR2**: Ryanodine Receptors, **SERCA2a**: Ca-ATPase of the SR, **PLN**: Phospholamban, **NCX**: $Na^{+}$-$Ca^{2+}$ exchanger, **PMCA**: plasma membrane $Ca^{2+}$ pump. For details see text.

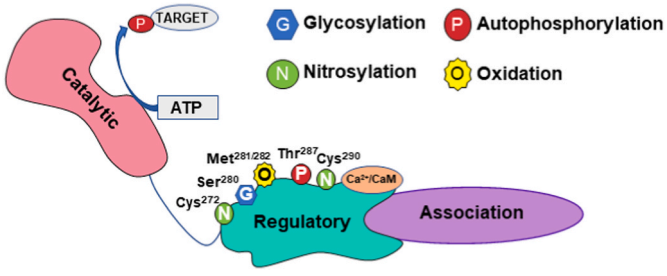


**Fig. 3.** CaMKII structure
The figure shows a schematic structure of the CaMKII monomer and different sites of CaMKII activation by different pathways (Glycosylation, Autophosphorylation, Oxidation and Nitrosylation. $Ca^{2+}$/CaM: calcium/Calmodulin. For details see text.

contribute to the genesis of cardiac arrhythmias in metabolic disorders. Among these mechanisms, CaMKII increased activity plays a central role. Indeed, in DM several cellular signaling pathways that produce physiological stress and may activate CaMKII are significantly altered (Jia et al., 2018).

In a recent review, Hegyi et al. (2019), discussed the involvement of CaMKII enhanced activity in several of the mechanisms of arrhythmias production during metabolic disturbances (described in Fig. 1) (Hegyi et al., 2019). We will concentrate here on the role of CaMKII on $Ca^{2+}$-triggered arrhythmias, *i.e.* arrhythmias directly associated with $Ca^{2+}$ handling alterations. However, it should be borne in mind that hyperglycemia and diabetes dysregulate different ion channels and produce a cardiac remodeling that contributes and/or acts as a substrate for the development of $Ca^{2+}$-triggered arrhythmias. CaMKII activity is at least partially responsive of this remodeling (Hegyi et al., 2021b).

### 1.4.1. *Preclinical studies*

An increase in pro-arrhythmogenic SR $Ca^{2+}$ leak/$Ca^{2+}$ sparks has been described in DC models of type 1 and 2 of diabetes mellitus (T1DM, T2DM) (Belke et al., 2004; Yaras et al., 2005). In Streptozotocin (STZ) treated rats (T1DM), Yaras et al. (2005) observed that the enhanced SR $Ca^{2+}$ leak occurs together with a decrease in RyR2 and FKBP12.6 expressions and an enhancement of RyR2 $Ser^{2809}$ site phosphorylation (Yaras et al., 2005). Shao et al. (2009), working in the same model supported the notion that SR $Ca^{2+}$ leak was due to CaMKII activation by showing that the phosphorylation of $Ser^{2808}$ and $Ser^{2814}$ RyR2 sites was increased in DC in association with an increase in CaMKII activity and a decrease in basal PKA activity, without changes in its maximum achievable activity (Shao et al., 2009). Stølen et al. (2009), simultaneously demonstrated that the increase in SR $Ca^{2+}$ leak in a T2DM model, the *db/db* mice, was associated to an increase in $Ser^{2814}$ site of RyR2 and of CaMKII activity. These authors also showed that there was a significant increase in $Thr^{17}$ phosphorylation of PLN, which might have been responsible for the sustained SR $Ca^{2+}$ leak observed in these mice (Stølen et al., 2009). Both Shao et al. (2009) and Stolen et al. (2009) associated, -for the first time to the best of our knowledge-, the CaMKII-induced increased SR $Ca^{2+}$ leak with the increased propensity to arrhythmias in DC (Stølen et al., 2009; Shao et al., 2009). Working STZ mice, Monnerat et al. (2016), described an increased inflammatory response, involving toll-like receptor 2 (TLR2) and NLRP3 inflammasome activation in cardiac macrophages with enhancement of interleukin 1 β (IL-1β) production (Monnerat et al., 2016). The authors further showed that treatment of isolated cardiomyocytes with IL-1β induced spontaneous contractile events that are associated with CaMKII oxidation and phosphorylation and were attenuated by CaMKII inhibition. The link between IL-1β and CaMKII oxidation was not explored in these experiments. A possible pathway suggested by the authors involves TLR and the myeloid differentiation protein 88 (MyD88), known to produce cardiomyocytes CaMKII oxidation in cardiac inflammation processes (Singh et al., 2012). On the other hand, experiments by Nishio et al. (2012) in STZ-induced diabetic rat hearts suggest that activation of CaMKII induced by impaired intracellular $Ca^{2+}$ metabolism stimulates reactive oxygen species (ROS) production in the diabetic heart associated with upregulation of NAPDH oxidase, which was prevented by KN-93 treatment, a selective CaMKII-inhibitor (Nishio et al., 2012).

Popescu et al., 2019, working with a rat model of late-onset T2DM (rats transgenic for human islet amyloid polypeptide (HIP) or HIP rats, (Butler et al., 2004)), showed both, a direct enhancement of RyR2 oxidation and RyR2 phosphorylation at $Ser^{2014}$ with a small but significant increase in $Ser^{2808}$ residue phosphorylation, associated with an increase in DADs. Interestingly, phosphorylation of $Ser^{2808}$ residue was again attributed to PKA phosphorylation without considering the possibility of CaMKII phosphorylation of the site. These authors also described an increase in phosphorylation of $Thr^{17}$ of PLN (the CaMKII site) and an increased intracellular $Na^{+}$ concentration which reduces $Ca^{2+}$ extrusion through NCX allowing SERCA to compete better for cytosolic $Ca^{2+}$, limiting the decrease in SR $Ca^{2+}$ load, and keeping it above the threshold for generating and maintaining SR $Ca^{2+}$ leak. (Popescu et al., 2019). Thus, direct RyR2 oxidation may add to phosphorylation to increase the gain of function of RyR2. Intriguingly, Tian et al. (2011) demonstrated an increase of RyR2 gain of function which was independent of phosphorylation and oxidation of the channels in

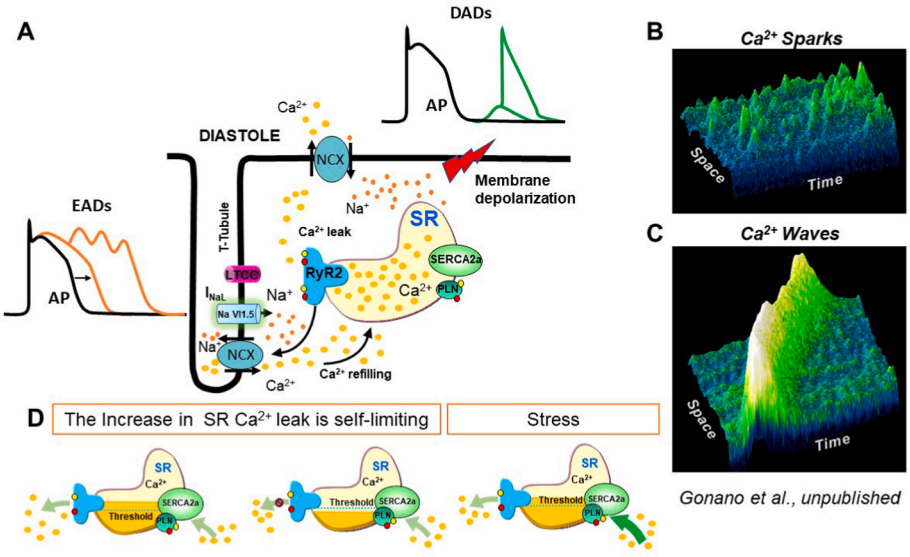


**Fig. 4.** *Early and Delay After- Depolarizations (EADs and DADs respectively)*
***A.*** Cartoon representing the mechanisms of EADs and DADs. The scheme shows how an increase in SR $Ca^{2+}$ leak propagates in the form of $Ca^{2+}$ waves throughout the cytosol, producing DADs and potentially an ectopic beat. A prolongation of the AP, due for instance to a prolongation of late Na current (INaL), would reduce the repolarization reserve and promote EADs. ***B and C:*** Fluorescence signals showing $Ca^{2+}$ sparks (***B***) and a $Ca^{2+}$ wave (***C***). **D.** Schematic representation of the importance of the exacerbated SR $Ca^{2+}$ reuptake to maintain SR $Ca^{2+}$ leak. To the left, the figure shows that under stress conditions, SR $Ca^{2+}$ can be recovered to reinstate SR $Ca^{2+}$ leak. AP: Action Potential. LTCC: L-type $Ca^{2+}$ channels, NCX: $Na^{+}$/$Ca^{2+}$ exchanger, RyR2: Ryanodine Receptors, PLN: Phospholamban, SR: Sarcoplasmic reticulum, SERCA2a: $Ca^{2+}$-ATPase of the SR, late component of the $Na^{+}$ current: INaL. Note that the excess $Na^{+}$ entering through the INaL is extruded from the cell through the NCX working in the reverse mode (extruding $Na^{+}$ and entering $Ca^{2+}$).

STZ-treated rats (Tian et al., 2011). These studies demonstrated that, besides CaMKII-dependent phosphorylation, several factors may influence the activity of RyR2.

Early studies associated an important role of O-GlcNAcylation of cytosolic and nuclear proteins with Cardiac dysfunction in T1DM and T2DM models (Fulop et al., 2007; Hu et al., 2005). Erickson et al. (2013) also associated the increase in CaMKII activity by O-GlcNAcylation with the enhanced SR $Ca^{2+}$ leak and ventricular arrhythmias produced by hyperglycemia. These authors demonstrated that the higher frequency of arrhythmias and the enhancement of CaMKII activity in diabetic rats could be prevented not only by CaMKII inhibition by KN-93 but also by the inhibition of O-GlcNAc transferase (OGT). Moreover, CaMKII-*O*-GlcNac expression was found to be doubled in HF patients and tripled in patients with HF and DM, promoting $Ca^{2+}$ mishandling (Erickson et al., 2013). Recently it was shown that O-GlcNAcylation of CaMKII at $Ser^{280}$ is the predominant mechanism for autonomous CaMKII activation in diabetic hyperglycemia. According to these results, oxidation of CaMKII at two neighboring methionine residues (281/2) appears to be mediated by an angiotensin II—NOX2 (NADPH oxidase 2) pathway, independently of hyperglycemia, although still playing a synergistic role in promoting autonomous kinase activity (Hegyi et al., 2021a, 2021b). In contrast, the same authors described an enhanced O-GlcNAcylation of PLN which may explain the reduced phosphorylation of $Ser^{16}$ site and the fact that $Thr^{17}$ residue phosphorylation does not change despite the increased activity of CaMKII. This combination would reduce SERCA2a activity, supporting the slowed relaxation observed in these diabetic rats. On the other hand, results by Mesubi et al. (2021) showed that oxidized CaMKII (Ox-CaMKII) is the primary mechanism that enhances triggered activity and RyR2 $Ca^{2+}$ leak in atrial fibrillation observed in mice models of T1DM and T2DM, while O-GlcNAcylation would act by CaMKII-independent mechanism(s) (Mesubi et al., 2021).

In summary, most of the experimental data support the notion that $Ca^{2+}$-triggered arrhythmias occur in DC and other metabolic disorders according to the mechanism schematized in Fig. 5A. This mechanism involves a CaMKII-dependent increased SR $Ca^{2+}$ leak which produces an enhancement of inward NCX current, which by depolarizing cell membrane promotes DADs and eventually ectopic beats. Direct post-translational modifications of RyR2, such as oxidation by ROS, may also contribute to altered RyR2 function as shown by different authors (Popescu et al., 2019; Tian et al., 2011). Of note, direct oxidation or CaMKII-induced phosphorylation of RyR2, promotes a vicious circle (in green) that contributes to maintain CaMKII activity and that may be strengthened by the phosphorylation of $Thr^{17}$ of PLN. Fig. 5B

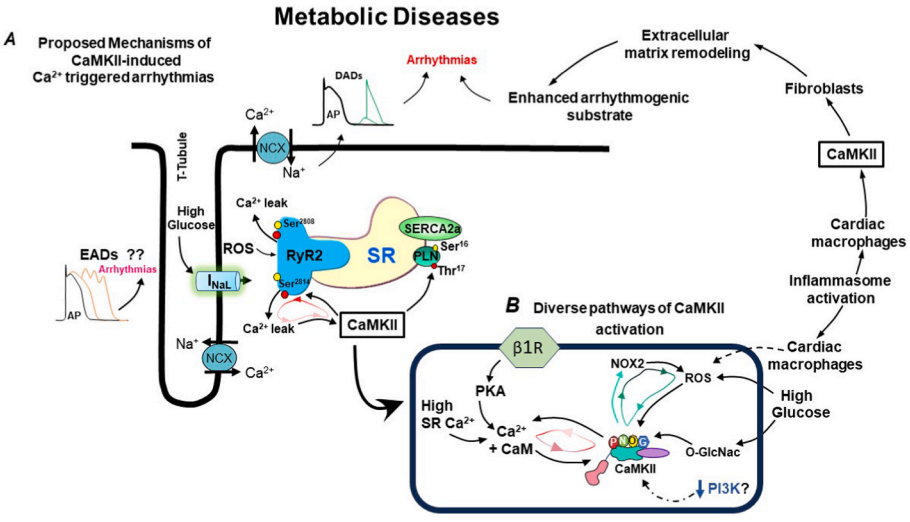


**Fig. 5.** Different pathways of CaMKII activation in metabolic diseases and proposed mechanisms of CaMKII-induced $Ca^{2+}$ triggered arrhythmias.
A. Scheme of the different mechanisms that may contribute to produce CaMKII-induced SR $Ca^{2+}$ leak and $Ca^{2+}$ triggered arrhythmias in metabolic diseases. B. Interaction among different pathways altered in metabolic diseases that produce CaMKII activation. Pink and green loops in the figures indicate the vicious circles formed by the enhanced SR $Ca^{2+}$ leak and the increased CaMKII activity which further enhance its activity and the increase on CaMKII-induced activity of NOX2 that by enhancing ROS production, further increase CaMKII activity, respectively. To the right, the contribution of CaMKII induced changes in the extracellular matrix to provide a substrate for $Ca^{2+}$-triggered arrhythmias, is indicated. Abbreviations like in Figs. 2 and 4. NOX2: NADPH oxidase 2, PI3K: PI 3 kinase 2. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

summarizes the different pathways compromised in DC and metabolic diseases that may converge to increase CaMKII activity by phosphorylation, glycosylation, and oxidation. The figure indicates the vicious circle mentioned above (CaMKII-RyR2-CaMKII) and a second vicious circle that occurred due to ROS-induced activation of CaMKII and subsequent NOX2 activation. Moreover, whether ROS or O-GlcNAcylation (both increased in DC) is the key mechanism for CaMKII increased activity, cardiac remodeling and arrhythmias in DC remains unsettled. The experimental evidence suggests that although the predominant mechanism may differ between diabetes types and stages of the disease, the final activity of CaMKII is the consequence of the synergistic action of different pathways, such as autophosphorylation, glycosylation and oxidation (Fig. 3).

So far, we have discussed mainly CaMKII-$Ca^{2+}$-triggered arrhythmias that involve DADs. However, it is important to mention that additional mechanisms also dependent on CaMKII contribute to, and even might be necessary for, the occurrence of $Ca^{2+}$-triggered arrhythmias in diabetic cardiac myocytes. For instance, recent experiments demonstrated that hyperglycemia is associated with an enhancement of INaL and a prolongation of the AP (Hegyi et al., 2021a). In this paper, the authors postulate that by reducing the repolarization reserve, the increase in INaL may constitute a second hit that greatly exacerbates diabetic hyperglycemia. As shown in Figs. 4 and 5A, a prolongation of INaL, previously reported in the ventricle of diabetic rats, may also evoke EADs that would contribute to triggered arrhythmias (Lu et al., 2013) The mechanism of this increase seems to be a defective phosphoinositide-3-kinase (PI3K) signaling pathway, which is linked to QT prolongation of the diabetic heart (Lu et al., 2013). Although the authors did not investigate a possible role of CaMKII in this signaling pathway, previous results strongly suggested that PI3K/AKT signaling may negatively modulate CaMKII activity by nitrogen monoxide (NO)-dependent S-nitrosylation (Sepúlveda et al., 2013). Thus, a defective PI3K signaling might favor CaMKII activity produced by another mechanism. However, CaMKII δ has two known sites for nitrosylation, $C^{290}$ and $C^{273}$. Whereas S-nitrosylation of $Cys^{290}$ on CaMKIIδ promotes kinase activity, S-nitrosylation of $Cys^{273}$ suppresses activation by $Ca^{2+}$–CaM (Erickson et al., 2015), which complicates the interpretation of these previous results. Further research is needed to evaluate the contribution of INaL increase in the enhanced triggered arrhythmias in the context of diabetes.

### 1.4.2. *Clinical findings*

As already discussed, cardiac arrhythmia propensity is a hallmark of DC, with an increased risk of sudden death (Movahed et al., 2007; Chaoul et al., 2023). Several altered pathways in DC may contribute to generate different types of arrhythmias (See for review Hegyi et al., 2019 (Hegyi et al., 2019)). In the context of this review, it is important to mention that autonomic-nervous-system dysfunction and activation of hormonal systems including the renin-angiotensin-aldosterone system was found to be associated with T2DM and metabolic syndrome (Carnethon et al., 2003; Brenner et al., 2001; Lindholm et al., 2002). These alterations contribute to increased oxidative stress and inflammation, which promote diabetic complications like cardiac arrhythmias and may involve autonomous CaMKII activation (Jungen et al., 2019; Romero-García et al., 2020). CaMKII may also be activated by ventricular remodeling which increases stress and strain on cardiac myocytes- This leads to the production of ROS and NO (Prosser et al., 2013; Jian et al., 2014). Inflammatory cytokines have been shown to induce cardiac arrhythmias in diabetic mice (Monnerat et al., 2016). Indeed, ox-CaMKII was found to be higher in diabetic patients than in non-diabetic patients (Luo et al., 2013), which is consistent with an increase in oxidative stress. Human atrial and ventricular samples from patients with T2DM also showed an increase in CaMKII O-GlcNAcylation (Erickson et al., 2013; Hegyi et al., 2021a). This is consistent with the excessive intracellular O-GlcNAcylation of multiple proteins that is seen in DM. Importantly, both chronic and acute diabetic hyperglycemia have been shown to activate CaMKII and arrhythmias (Erickson et al., 2013). Indeed hyperglycemia is associated with an increase in the risk of arrhythmias in patients with DM (Hagelqvist et al., 2023).

Prolongation of heart rate-corrected QT-interval (QTc) is frequently observed in diabetic patients (Ninkovic et al., 2016). This prolongation has been correlated with the increased AP duration in diabetic myocytes, which is generally associated with a decrease in $K^+$ currents (Zhang et al., 2007; Meo et al., 2016). However, remodeling of other ionic currents may also be involved in AP and QTc prolongation in diabetes, as discussed above.

Taken together, pre-clinical and clinical studies support a central role of CaMKII kinase in the exacerbated propensity to arrhythmias in DC.

### 1.4.3. *Non-myocyte cells may contribute to $Ca^{2+}$-triggered arrhythmias in metabolic disorders*

Cardiac fibroblasts, the coronary vasculature, the autonomic nervous system, and even immune cells comprise a great part of the complex cardiac architecture and contribute to orchestrate cardiac function (Litviňuková et al., 2020). Emerging evidence suggests that these non-myocyte cells may play a significant role in the development of $Ca^{2+}$-triggered arrhythmias during metabolic disorders. Although the role of these cells is out of the scope of this review, we feel that it is important to make a brief discussion about their possible contribution to cardiac remodeling and arrhythmias, since their importance is often overlooked in preclinical models of DC and other metabolic alterations.

Cardiac fibroblasts, traditionally recognized for their structural support function in the myocardium, undergo phenotypic changes in metabolic disorders. In response to hyperglycemia, fibroblasts upregulate and remodel cardiac extracellular matrix components (Frangogiannis, 2019), causing contractile dysfunction and fibrosis. These changes would contribute to the necessary arrhythmogenic substrate for arrhythmias to occur. However, and as we will discuss below, $Ca^{2+}$-triggered arrhythmias occur in prediabetic models in the absence of evident fibrosis (Sommese et al., 2016; Federico et al., 2017). The pro-fibrotic activity of fibroblasts and myofibroblasts is further exacerbated by growth factors such as transforming growth factor β-1 (TGFβ-1), connective tissue growth factor, and various interleukins, all of which are upregulated in the diabetic myocardium (Souders et al., 2009). Furthermore, immune cells, including macrophages and lymphocytes, are increasingly recognized as important contributors to cardiac remodeling and inflammation during metabolic disorders (Lazzerini et al., 2018). These immune cells can release pro-inflammatory cytokines, activate signaling pathways involved in $Ca^{2+}$ handling, and induce oxidative stress, all of which may disturb $Ca^{2+}$ homeostasis and promote arrhythmia susceptibility. For example, TNF-α and IL-1β increase $Ca^{2+}$ leak from the SR, which contributes to depressed systolic $Ca^{2+}$ transients, resulting in cardiac contractile dysfunction and arrhythmias in rat ventricular myocytes (Lazzerini et al., 2018; Arbel, 2018). Dysfunction of endothelial cells in metabolic disorders can impair coronary blood flow and nutrient supply, further exacerbating $Ca^{2+}$ dysregulation and promoting arrhythmogenesis (de Zeeuw et al., 2015; Jamwal and Sharma, 2018).

The role of CaMKII in interstitial fibrosis is well-established in several cardiomyopathies (Willeford et al., 2018). It is known that CaMKII inhibition attenuates the inflammatory response and fibrosis by influencing NFκB and MAPK signaling following I/R injury (Gray et al., 2017; Ling et al., 2013). Thus, increased CaMKII activity may also be involved in the mechanism by which non -myocytes cardiac cells contribute to $Ca^{2+}$-triggered arrhythmias. Fig. 5B schematizes this possibility. Understanding the complex interactions among various cardiac cell types in the context of metabolic disorders is crucial for unraveling the intricate mechanisms underlying $Ca^{2+}$-triggered arrhythmias and identifying potential therapeutic targets beyond cardiac myocytes.

### 1.5. CaMKII and arrhythmias at the earlier stages of DM and metabolic syndrome

Surprisingly, despite the acknowledgment of the prolonged evolution of DC and the rising prevalence of prediabetes (Lin et al., 2018; Shang et al., 2019), studies tracking the early stages of DC are scarce.

In a mouse model of prediabetes (high fructose rich diet), Sommese et al. (2016), found an increase in ox- CaMKII activity in association with an increase in RyR2 and PLN phosphorylation at the CaMKII sites ($Ser^{2814}$ and $Thr^{17}$, respectively), and enhancement of SERCA2a activity (Sommese et al., 2016). The increase in RyR2 and PLN phosphorylation were associated with increases in SR $Ca^{2+}$ leak, spontaneous contractions, and arrhythmias. All of these spontaneous phenomena could be prevented by the CaMKII inhibitor KN-93, the ROS scavenger TEMPOL, and also in S2814A mice (Chelu et al., 2009; Van Oort et al., 2010), in which the CaMKII site of the RyR2 is replaced by Ala, an amino acid that cannot be phosphorylated. They were also prevented in SR-AIP mice (Ji et al., 2003), in which the CaMKII inhibitor autocamtide inhibitory peptide (AIP) is targeted to the SR (Sommese et al., 2016). These results indicated that the increase in ROS production activates CaMKII, increasing RyR2 and PLN phosphorylation, which enhances and maintains SR $Ca^{2+}$ leak. Although in these experiments RyR2 oxidation was not explored in these experiments, their possible contribution to arrhythmias can be discarded, since they were prevented by CaMKII inhibition in the presence of increased ROS levels.

Obesity and dietary saturated fat also promote arrhythmias associated to CaMKII. In mice fed a high saturated diet, Joseph et al. (2019, 2021) demonstrated an increase in ventricular ectopy susceptibility to induced ventricular tachycardia (Joseph et al., 2019, 2021). The electrophysiological abnormalities appeared before the onset of obesity and were associated with an increase in oxidative stress resulting from the activation of NOX2. Oxidation of RyR2 could enhance SR $Ca^{2+}$ leak with a consequent increase in CaMKII which would further enhance SR $Ca^{2+}$ leak (Joseph et al., 2019). The authors further associated the increase in SR $Ca^{2+}$ leak with mitochondrial $Ca^{2+}$ overload, since mitochondrial $Ca^{2+}$ uniporter (MCU) deletion prevents ventricular arrhythmias, which were also prevented by CaMKII inhibition (Joseph et al., 2021).

Sánchez et al. (2018) found an association between redox modifications of RyR2 and arrhythmias in mice treated with a high-fat diet. Although no exploration of CaMKII activity or CaMKII-dependent phosphorylation of RyR2 was performed in this study, the results indicated that the increase in ROS activity that occurs in metabolic disorders may directly affect the redox state of different ECC proteins, including RyR2, and be responsible at least in part of the arrhythmias observed (Sánchez et al., 2018).

The role of CaMKII has also been studied in rats with MS. Specifically, CaMKII autonomously activated was shown to be a contributing factor to the mechanism of abnormal diastolic $Ca^{2+}$ leak, which can be further exacerbated under β-adrenergic stimulation (Romero-García et al., 2020). In recent experiments conducted on isolated cardiomyocytes, it was shown that exposure to high levels of extracellular glucose leads to an increase in O-GlcNac modification on CaMKII, which activates NOX2 and increases the production of ROS, initiating a self-perpetuating vicious cycle. As a result, CaMKII can be also activated by oxidation, increasing SR $Ca^{2+}$ leak (Hegyi et al., 2021c). Moreover, experiments performed in mice that develop mild hyperglycemia and obesity after treatment with low-dose STZ and high-fat diet, have shown that O-GlcNAc levels increase as early as two months post-treatment (Fricovsky et al., 2012). Taken together, these data suggest that CaMKII-*O*-GlcNac may also be an early phenomenon in the evolution of DC.

The findings observed in obese and prediabetic models are of critical importance. Because cardiac defects in DM have a long progression before reaching the stage of DC with evident diastolic and systolic symptoms, knowledge of cellular and subcellular alterations occurring at the early stages of the disease is necessary and essential for setting the first steps in the evolution of this cardiac illness at this level. This knowledge may pave the way for the development of early diagnostic tools and preventive interventions, ultimately improving patient outcomes and reducing the burden of cardiovascular disease in this vulnerable population.

## 2. Therapeutic approaches and future challenges

Although the association of DC and metabolic disorders with increased arrhythmia risk is well established, effective therapies are still lacking (Cosentino et al., 2020). Due to the key role played by CaMKII, targeting this kinase has emerged as a potential and promising therapeutic strategy to prevent or mitigate these types of arrhythmias. Indeed, as mentioned above, CaMKII inhibition has been found to be protective against arrhythmias in various diabetic animal models (Chelu et al., 2009; Carter et al., 2006; Kushnir et al., 2018; Marx et al., 2000; Sommese et al., 2016; Hegyi et al., 2021b; Popescu et al., 2019; Kadosaka et al., 2023). However, a significant gap remains between advances at the bench and new treatments in human patients. In recent years, efforts were made to develop new pharmacologic drugs for CaMKII inhibition, based on the pyrimidine ATP-competitive class of CaMKII inhibitors such as AS105 developed by Allosteros Therapeutics (Neef et al., 2018) or GS-680, from Gilead Sciences (Lebek et al., 2023). The most recently designed compound named RA306 developed by Sanofi R&D (Beauverger et al., 2020), was found to be a potent inhibitor of human CaMKII δ and γ, the two main cardiac isoforms and has the unique characteristic of being an orally bioavailable agent, giving it a significant advantage for potential clinical investigation. A recent study tested the potential inhibitory action of a large number of compounds orally bioavailable and approved by the U.S. Food and Drug Administration. This yielded five previously unknown CaMKII inhibitors with clinically relevant potency: ruxolitinib, baricitinib, silmitasertib, crenolanib, and abemaciclib. It was found that ruxolitinib abolished arrhythmogenesis in mouse and patient-derived models of CaMKII-driven arrhythmias (Reyes Gaido et al., 2023).

Peptide inhibitors of CaMKII offer several advantages over small molecules but face many challenges for human delivery. A relatively recent study showed that biocompatible and biodegradable calcium phosphate nanoparticles introduced by inhalation can be used to successfully deliver peptide cargo to the heart (Miragoli et al., 2018). This technique represents a potentially robust opportunity for delivering peptide inhibitors.

Interfering RNA/DNAs have been widely used as a research tool, but their toxicity, off-target effects, and short half-lives due to nucleases activity prevented their clinical use. Recent improvements have overcome these difficulties, and an antisense nucleotide (ASO) against CaMKIIδ from Ionis Pharmaceuticals have been shown to reduce cardiac CaMKII and arrhythmic events in mice that had undergone myocardial infarction (Yeh et al., 2020).

The use of CRISPR-Cas9 ABE system to edit the genome overcomes many of the limitations of previously described tools to target CaMKII. Lebek et al. (2023) recently showed that editing the CaMKIIδ gene to eliminate oxidation-sensitive methionine residues protects cardiomyocytes derived from human-induced pluripotent stem cells from ischemia/reperfusion injury, providing a novel and promising technique to permanently change the CaMKIIδ gene sequency (Lebek et al., 2023). Fig. 6 summarizes the different possible therapeutic approaches developed to inhibit CaMKII.

Another focus of current research and potential clinical therapies is to prevent the consequences of hyperglycemia and oxidative stress, which include post-translational modifications of CaMKII and its protein targets. Interestingly, recent experiments have shown a link between acute high glucose and the promotion of both, ROS production and CaMKII activation, forming an arrhythmogenic vicious cycle (Hegyi et al., 2021c). In connection with this, empagliflozin (EMPA), a drug that reduces both ROS and O-GlcNAc levels in diabetic hearts, significantly attenuated arrhythmia susceptibility and Ca2+ alterations in a

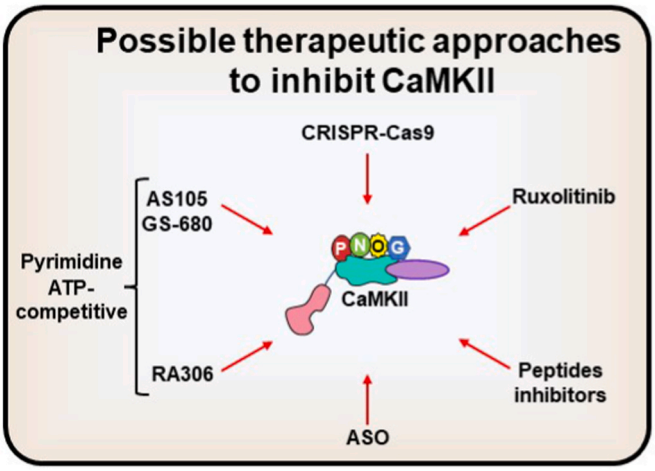


**Fig. 6.** Different therapeutic approaches tested to inhibit CaMKII.
Different therapeutic approaches were used in an attempt to inhibit CaMKII: See text for further explanation. ASO: Antisense oligonucleotides.

mouse model of T2DM. (Kadosaka et al., 2023). Although the effects of empagliflozin reveal a potential novel mechanism of action, the exact molecular target(s) of empagliflozin in cardiac myocytes is still to be determined.

While the importance of CaMKII inhibition in metabolic disorders and arrhythmias is becoming clear, further research is required to optimize the therapeutic strategies. A crucial challenge is to develop selective and safe CaMKII inhibitors that specifically target the cardiac isoform of CaMKII and do not interfere with other essential cellular functions. Additionally, clinical trials are needed to evaluate the efficacy and long-term safety of CaMKII inhibition in patients with diabetic cardiomyopathy and other metabolic disorders.

## 3. Concluding remarks

Strong experimental evidence indicates that the increased activity of CaMKII is one of the prevailing mechanisms involved in $Ca^{2+}$ mishandling that can lead to triggered arrhythmias in different pathologies, including metabolic disorders. The mechanistic understanding of CaMKII signaling, and arrhythmia mechanisms may reveal new therapeutic targets and ultimately better treatment of DC and associated metabolic diseases. More important, these alterations can be detected at the very early stages of the progression to DC. These studies underscore the need for timely detection of the disease at the possibly unique stages at which reversal of $Ca^{2+}$ handling alterations could be achieved, and sudden cardiac death could be prevented.

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## Declaration of competing interest

None

## References

Ai, X., Curran, J.W., Shannon, T.R., Bers, D.M., Pogwizd, S.M., 2005. Ca2+/calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca2+ leak in heart failure. Circ. Res. 97, 1314–1322. https://doi.org/10.1161/01.RES.0000194329.41863.89.

Anderson, M.E., Braun, A.P., Schulman, H., Premack, B.A., 1994. Multifunctional Ca2+/calmodulin-dependent protein kinase mediates Ca (2+)-induced enhancement of the L-type Ca2+ current in rabbit ventricular myocytes. Circ. Res. 75, 854–861.

Anderson, M.E., Brown, J.H., Bers, D.M., 2011. CaMKII in myocardial hypertrophy and heart failure. J. Mol. Cell. Cardiol. 51, 468–473. https://doi.org/10.1016/j.yjmcc.2011.01.012.

Arbel, Y.S.Y., 2018. A Review of Interleukin-1 in Heart Disease: where Do We Stand Today? Cardiol. Ther. 7, 25–44.

Arvanitis, D.A., Vafiadaki, E., Johnson, D.M., Kranias, E.G., Sanoudou, D., 2018. The histidine-rich calcium binding protein in regulation of cardiac rhythmicity. Front. Physiol. 9, 1379. https://doi.org/10.3389/fphys.2018.01379.

Backs, J., Backs, T., Neef, S., Kreusser, M.M., Lehmann, L.H., Patrick, D.M., Grueter, C.E., Qi, X., Richardson, J.A., Hill, J.A., Katus, H.A., Bassel-Duby, R., Maier, L.S., Olson, E. N., 2009. The δ isoform of CaM kinase II is required for pathological cardiac hypertrophy and remodeling after pressure overload. Proc. Natl. Acad. Sci. USA 106, 2342–2347. https://doi.org/10.1073/pnas.0813013106.

Beauverger, P., Ozoux, M.-L., Bégis, G., Glénat, V., Briand, V., Philippo, M.-C., Daveu, C., Tavares, G., Roy, S., Corbier, A., Briand, P., Dorchies, O., Bauchet, A.-L., Nicolai, E., Duclos, O., Tamarelle, D., Pruniaux, M.-P., Muslin, A.J., Janiak, P., 2020. Reversion of cardiac dysfunction by a novel orally available calcium/calmodulin-dependent protein kinase II inhibitor, RA306, in a genetic model of dilated cardiomyopathy. Cardiovasc. Res. 116, 329–338. https://doi.org/10.1093/cvr/cvz097.

Belke, D.D., Swanson, E.A., Dillmann, W.H., 2004. Decreased sarcoplasmic reticulum activity and contractility in diabetic db/db mouse heart. Diabetes 53, 3201–3208.

Bers, D.M., 2002. Cardiac excitation-contraction coupling. Nature 415, 198–205. https://doi.org/10.1038/415198a.

Bossuyt, J., Helmstadter, K., Wu, X., Clements-Jewery, H., Haworth, R.S., Avkiran, M., Martin, J.L., Pogwizd, S.M., Bers, D.M., 2008. Ca2+/calmodulin-dependent protein kinase IIdelta and protein kinase D overexpression reinforce the histone deacetylase 5 redistribution in heart failure. Circ. Res. 102, 695–702. https://doi.org/10.1161/CIRCRESAHA.107.169755.

Braun, A.P., Schulman, H., 1995. The multifunctional calcium/calmodulin-dependent protein kinase: from form to function. Annu. Rev. Physiol. 57, 417–445.

Brenner, B.M., Cooper, M.E., De Zeeuw, D., Keane, W.F., Mitch, W.E., Parving, H.-H., Remuzzi, G., Snapinn, S.M., Zhang, Z., Shahinfar, S., 2001. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N. Engl. J. Med. 345, 861–869.

Butler, A.E., Jang, J., Gurlo, T., Carty, M.D., Soeller, W.C., Butler, P.C., 2004. Diabetes due to a progressive defect in β-cell mass in rats transgenic for human islet amyloid polypeptide (HIP rat) a new model for type 2 diabetes. Diabetes 53, 1509–1516.

Campbell, H.M., Quick, A.P., Abu-Taha, I., Chiang, D.Y., Kramm, C.F., Word, T.A., Brandenburg, S., Hulsurkar, M., Alsina, K.M., Liu, H.-B., Martin, B., Uhlenkamp, D., Moore, O.M., Lahiri, S.K., Corradini, E., Kamler, M., Heck, A.J.R., Lehnart, S.E., Dobrev, D., Wehrens, X.H.T., 2020. Loss of SPEG inhibitory phosphorylation of RyR2 promotes atrial fibrillation. Circulation 142, 1159–1172. https://doi.org/10.1161/CIRCULATIONAHA.120.045791.

Carnethon, M.R., Golden, S.H., Folsom, A.R., Haskell, W., Liao, D., 2003. Prospective investigation of autonomic nervous system function and the development of type 2 diabetes: the Atherosclerosis Risk in Communities study, 1987-1998. Circulation 107, 2190–2195. https://doi.org/10.1161/01.CIR.0000066324.74807.95.

Carter, S., Colyer, J., Sitsapesan, R., 2006. Maximum phosphorylation of the cardiac ryanodine receptor at serine-2809 by protein kinase a produces unique modifications to channel gating and conductance not observed at lower levels of phosphorylation. Circ. Res. 98, 1506–1513.

Chan, Y.-H., Chang, G.-J., Lai, Y.-J., Chen, W.-J., Chang, S.-H., Hung, L.-M., Kuo, C.-T., Yeh, Y.-H., 2019. Atrial fibrillation and its arrhythmogenesis associated with insulin resistance. Cardiovasc. Diabetol. 18, 1–14.

Chaoul, V., Hanna, R., Hachem, P., El Hayek, M.S., Nour-Eldine, W., Abou-Khalil, P., Abi-Ramia, E., Vandecasteele, G., Abi-Gerges, A., 2023. Differential changes in cyclic adenosine 3′-5′ monophosphate (cAMP) effectors and major Ca2+ handling proteins during diabetic cardiomyopathy. J. Cell Mol. Med. 27, 1277–1289.

Chelu, M.G., Sarma, S., Sood, S., Wang, S., Van Oort, R.J., Skapura, D.G., Li, N., Santonastasi, M., Müller, F.U., Schmitz, W., 2009. Calmodulin kinase II–mediated sarcoplasmic reticulum Ca 2+ leak promotes atrial fibrillation in mice. J. Clin. Investig. 119, 1940–1951.

Cheng, H., Lederer, W.J., Cannell, M.B., 1993. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. Science 262, 740–744.

Cho, N.H., Shaw, J., Karuranga, S., Huang, Y., da Rocha Fernandes, J., Ohlrogge, A., Malanda, B., 2018. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res. Clin. Pract. 138, 271–281.

Cosentino, F., Grant, P.J., Aboyans, V., Bailey, C.J., Ceriello, A., Delgado, V., Federici, M., Filippatos, G., Grobbee, D.E., Hansen, T.B., Huikuri, H.V., Johansson, I., Jüni, P., Lettino, M., Marx, N., Mellbin, L.G., Östgren, C.J., Rocca, B., Roffi, M., Sattar, N., Seferović, P.M., Sousa-Uva, M., Valensi, P., Wheeler, D.C., 2020. ESC Scientific Document Group, 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur. Heart J. 41, 255–323. https://doi.org/10.1093/eurheartj/ehz486.

de la Bastie, D., Levitsky, D., Rappaport, L., Mercadier, J.J., Marotte, F., Wisnewsky, C., Brovkovich, V., Schwartz, K., Lompré, A.M., 1990. Function of the sarcoplasmic reticulum and expression of its Ca2(+)-ATPase gene in pressure overload-induced cardiac hypertrophy in the rat. Circ. Res. 66, 554–564. https://doi.org/10.1161/01.res.66.2.554.

de Zeeuw, P., Wong, B.W., Carmeliet, P., 2015. Metabolic adaptations in diabetic endothelial cells. Circ. J. 79, 934–941.

Di Carlo, M.N., Said, M., Ling, H., Valverde, C.A., De Giusti, V.C., Sommese, L., Palomeque, J., Aiello, E.A., Skapura, D.G., Rinaldi, G., Respress, J.L., Brown, J.H.,

Wehrens, X.H.T., Salas, M.A., Mattiazzi, A., 2014. CaMKII-dependent phosphorylation of cardiac ryanodine receptors regulates cell death in cardiac ischemia/reperfusion injury. J. Mol. Cell. Cardiol. 74, 274–283. https://doi.org/10.1016/j.yjmcc.2014.06.004.

Erickson, J.R., Joiner, M.A., Guan, X., Kutschke, W., Yang, J., Oddis, C.V., Bartlett, R.K., Lowe, J.S., O'Donnell, S.E., Aykin-Burns, N., Zimmerman, M.C., Zimmerman, K., Ham, A.-J.L., Weiss, R.M., Spitz, D.R., Shea, M.A., Colbran, R.J., Mohler, P.J., Anderson, M.E., 2008. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. Cell 133, 462–474. https://doi.org/10.1016/j.cell.2008.02.048.

Erickson, J.R., Pereira, L., Wang, L., Han, G., Ferguson, A., Dao, K., Copeland, R.J., Despa, F., Hart, G.W., Ripplinger, C.M., Bers, D.M., 2013. Diabetic hyperglycaemia activates CaMKII and arrhythmias by O-linked glycosylation. Nature 502, 372–376. https://doi.org/10.1038/nature12537.

Erickson, J.R., Nichols, C.B., Uchinoumi, H., Stein, M.L., Bossuyt, J., Bers, D.M., 2015. S-nitrosylation induces both autonomous activation and inhibition of calcium/calmodulin-dependent protein kinase II δ. J. Biol. Chem. 290, 25646–25656. https://doi.org/10.1074/jbc.M115.650234.

Federico, M., Portiansky, E.L., Sommese, L., Alvarado, F.J., Blanco, P.G., Zanuzzi, C.N., Dedman, J., Kaetzel, M., Wehrens, X.H.T., Mattiazzi, A., Palomeque, J., 2017. Calcium-calmodulin-dependent protein kinase mediates the intracellular signalling pathways of cardiac apoptosis in mice with impaired glucose tolerance. J. Physiol. 595, 4089–4108. https://doi.org/10.1113/JP273714.

Federico, M., Valverde, C.A., Mattiazzi, A., Palomeque, J., 2020. Unbalance between sarcoplasmic reticulum Ca2 + uptake and release: a first step toward Ca2 + triggered arrhythmias and cardiac damage. Front. Physiol. 10 https://doi.org/10.3389/fphys.2019.01630.

Ferrero, P., Said, M., Sánchez, G., Vittone, L., Valverde, C., Donoso, P., Mattiazzi, A., Mundiña-Weilenmann, C., 2007. Ca2+/calmodulin kinase II increases ryanodine binding and Ca2+-induced sarcoplasmic reticulum Ca2+ release kinetics during β-adrenergic stimulation. J. Mol. Cell. Cardiol. 43, 281–291.

Frangogiannis, N.G., 2019. The extracellular matrix in ischemic and nonischemic heart failure. Circ. Res. 125, 117–146.

Fricovsky, E.S., Suarez, J., Ihm, S.-H., Scott, B.T., Suarez-Ramirez, J.A., Banerjee, I., Torres-Gonzalez, M., Wang, H., Ellrott, I., Maya-Ramos, L., 2012. Excess protein O-GlcNAcylation and the progression of diabetic cardiomyopathy. Am. J. Physiol. Regul. Integr. Comp. Physiol. 303, R689–R699.

Fulop, N., Mason, M.M., Dutta, K., Wang, P., Davidoff, A.J., Marchase, R.B., Chatham, J.C., 2007. Impact of Type 2 diabetes and aging on cardiomyocyte function and O-linked N-acetylglucosamine levels in the heart. Am. J. Physiol. Cell Physiol. 292, C1370–C1378.

Gonano, L.A., Sepúlveda, M., Rico, Y., Kaetzel, M., Valverde, C.A., Dedman, J., Mattiazzi, A., Vila Petroff, M., 2011. Calcium-calmodulin kinase II mediates digitalis-induced arrhythmias. Circ. Arrhythmia Electrophysiol. 4, 947–957. https://doi.org/10.1161/CIRCEP.111.964908.

Gray, C.B., Suetomi, T., Xiang, S., Mishra, S., Blackwood, E.A., Glembotski, C.C., Miyamoto, S., Westenbrink, B.D., Brown, J.H., 2017. CaMKIIδ subtypes differentially regulate infarct formation following ex vivo myocardial ischemia/reperfusion through NF-κB and TNF-α. J. Mol. Cell. Cardiol. 103, 48–55.

Grisanti, L.A., 2018. Diabetes and arrhythmias: pathophysiology, mechanisms and therapeutic outcomes. Front. Physiol. 9, 1669.

Hagelqvist, P.G., Andersen, A., Maytham, K., Andreasen, C.R., Engberg, S., Lindhardt, T.B., Forman, J.L., Pedersen-Bjergaard, U., Knop, F.K., Vilsbøll, T., 2023. Glycaemia and cardiac arrhythmias in people with type 1 diabetes: a prospective observational study. Diabetes Obes. Metabol. 25, 2300–2309. https://doi.org/10.1111/dom.15108.

Hegyi, B., Bers, D.M., Bossuyt, J., 2019. CaMKII signaling in heart diseases: emerging role in diabetic cardiomyopathy. J. Mol. Cell. Cardiol. 127, 246–259.

Hegyi, B., Ko, C.Y., Bossuyt, J., Bers, D.M., 2021a. Two-hit mechanism of cardiac arrhythmias in diabetic hyperglycemia: reduced repolarization reserve, neurohormonal stimulation and heart failure exacerbate susceptibility. Cardiovasc. Res. https://doi.org/10.1093/cvr/cvab006.

Hegyi, B., Fasoli, A., Ko, C.Y., Van, B.W., Alim, C.C., Shen, E.Y., Ciccozzi, M.M., Tapa, S., Ripplinger, C.M., Erickson, J.R., 2021b. CaMKII serine 280 O-GlcNAcylation links diabetic hyperglycemia to proarrhythmia. Circ. Res. 129, 98–113.

Hegyi, B., Pölönen, R.-P., Hellgren, K.T., Ko, C.Y., Ginsburg, K.S., Bossuyt, J., Mercola, M., Bers, D.M., 2021c. Cardiomyocyte Na+ and Ca2+ mishandling drives vicious cycle involving CaMKII, ROS, and ryanodine receptors. Basic Res. Cardiol. 116, 58. https://doi.org/10.1007/s00395-021-00900-9.

Hu, Y., Belke, D., Suarez, J., Swanson, E., Clark, R., Hoshijima, M., Dillmann, W.H., 2005. Adenovirus-mediated overexpression of O-GlcNAcase improves contractile function in the diabetic heart. Circ. Res. 96, 1006–1013.

Ishida, A., Shigeri, Y., Taniguchi, T., Kameshita, I., 2003. Protein phosphatases that regulate multifunctional Ca2+/calmodulin-dependent protein kinases: from biochemistry to pharmacology. Pharmacol. Therapeut. 100, 291–305.

Jamwal, S., Sharma, S., 2018. Vascular endothelium dysfunction: a conservative target in metabolic disorders. Inflamm. Res. 67, 391–405.

Ji, Y., Li, B., Reed, T.D., Lorenz, J.N., Kaetzel, M.A., Dedman, J.R., 2003. Targeted inhibition of Ca2+/calmodulin-dependent protein kinase II in cardiac longitudinal sarcoplasmic reticulum results in decreased phospholamban phosphorylation at threonine 17. J. Biol. Chem. 278, 25063–25071.

Jia, G., Hill, M.A., Sowers, J.R., 2018. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. Circ. Res. 122, 624–638.

Jian, Z., Han, H., Zhang, T., Puglisi, J., Izu, L.T., Shaw, J.A., Onofiok, E., Erickson, J.R., Chen, Y.-J., Horvath, B., 2014. Mechanochemotransduction during cardiomyocyte contraction is mediated by localized nitric oxide signaling. Sci. Signal. 7 ra27–ra27.

Joseph, L.C., Avula, U.M.R., Wan, E.Y., Reyes, M.V., Lakkadi, K.R., Subramanyam, P., Nakanishi, K., Homma, S., Muchir, A., Pajvani, U.B., 2019. Dietary saturated fat promotes arrhythmia by activating NOX2 (NADPH Oxidase 2). Circulation: Arrhythmia Electrophysiol. 12, e007573.

Joseph, L.C., Reyes, M.V., Homan, E.A., Gowen, B., Avula, U.M.R., Goulbourne, C.N., Wan, E.Y., Elrod, J.W., Morrow, J.P., 2021. The mitochondrial calcium uniporter promotes arrhythmias caused by high-fat diet. Sci. Rep. 11, 17808.

Jungen, C., Scherschel, K., Flenner, F., Jee, H., Rajendran, P., De Jong, K.A., Nikolaev, V., Meyer, C., Ardell, J.L., Tompkins, J.D., 2019. Increased arrhythmia susceptibility in type 2 diabetic mice related to dysregulation of ventricular sympathetic innervation. Am. J. Physiol. Heart Circ. Physiol. 317, H1328–H1341. https://doi.org/10.1152/ajpheart.00249.2019.

Kadosaka, T., Watanabe, M., Natsui, H., Koizumi, T., Nakao, M., Koya, T., Hagiwara, H., Kamada, R., Temma, T., Karube, F., Fujiyama, F., Anzai, T., 2023. Empagliflozin attenuates arrhythmogenesis in diabetic cardiomyopathy by normalizing intracellular Ca2+ handling in ventricular cardiomyocytes. Am. J. Physiol. Heart Circ. Physiol. 324, H341–H354. https://doi.org/10.1152/ajpheart.00391.2022.

Kannel, W.B., Hjortland, M., Castelli, W.P., 1974. Role of diabetes in congestive heart failure: the Framingham study. Am. J. Cardiol. 34, 29–34.

Kranias, E.G., Hajjar, R.J., 2012. Modulation of cardiac contractility by the phospholamban/SERCA2a regulatome. Circ. Res. 110, 1646–1660. https://doi.org/10.1161/CIRCRESAHA.111.259754.

Kushnir, A., Wajsberg, B., Marks, A.R., 2018. Ryanodine receptor dysfunction in human disorders. Biochim. Biophys. Acta Mol. Cell Res. 1865, 1687–1697.

Lascano, E.C., Said, M., Vittone, L., Mattiazzi, A., Mundiña-Weilenmann, C., Negroni, J.A., 2013. Role of CaMKII in post acidosis arrhythmias: a simulation study using a human myocyte model. J. Mol. Cell. Cardiol. 60, 172–183. https://doi.org/10.1016/j.yjmcc.2013.04.018.

Laurita, K.R., Rosenbaum, D.S., 2008. Mechanisms and potential therapeutic targets for ventricular arrhythmias associated with impaired cardiac calcium cycling. J. Mol. Cell. Cardiol. 44, 31–43.

Lazzerini, P.E., Capecchi, P.L., El-Sherif, N., Laghi-Pasini, F., Boutjdir, M., 2018. Emerging arrhythmic risk of autoimmune and inflammatory cardiac channelopathies. J. Am. Heart Assoc. 7, e010595.

Lebek, S., Chemello, F., Caravia, X.M., Tan, W., Li, H., Chen, K., Xu, L., Liu, N., Bassel-Duby, R., Olson, E.N., 2023. Ablation of CaMKIIδ oxidation by CRISPR-Cas9 base editing as a therapy for cardiac disease. Science 379, 179–185. https://doi.org/10.1126/science.ade1105.

Lin, J., Thompson, T.J., Cheng, Y.J., Zhuo, X., Zhang, P., Gregg, E., Rolka, D.B., 2018. Projection of the future diabetes burden in the United States through 2060. Popul. Health Metrics 16, 1–9.

Lindemann, J.P., Watanabe, A.M., 1985. Phosphorylation of phospholamban in intact myocardium. Role of Ca2+-calmodulin-dependent mechanisms. J. Biol. Chem. 260, 4516–4525.

Lindholm, L.H., Ibsen, H., Borch-Johnsen, K., Olsen, M.H., Wachtell, K., Dahlöf, B., Devereux, R.B., Beevers, G., de Faire, U., Fyhrquist, F., 2002. Risk of new-onset diabetes in the Losartan Intervention for Endpoint reduction in hypertension study. J. Hypertens. 20, 1879–1886.

Ling, H., Gray, C.B., Zambon, A.C., Grimm, M., Gu, Y., Dalton, N., Purcell, N.H., Peterson, K., Brown, J.H., 2013. Ca2+/Calmodulin-dependent protein kinase II δ mediates myocardial ischemia/reperfusion injury through nuclear factor-κB. Circ. Res. 112, 935–944.

Litviňuková, M., Talavera-López, C., Maatz, H., Reichart, D., Worth, C.L., Lindberg, E.L., Kanda, M., Polanski, K., Heinig, M., Lee, M., Nadelmann, E.R., Roberts, K., Tuck, L., Fasouli, E.S., DeLaughter, D.M., McDonough, B., Wakimoto, H., Gorham, J.M., Samari, S., Mahbubani, K.T., Saeb-Parsy, K., Patone, G., Boyle, J.J., Zhang, H., Zhang, H., Viveiros, A., Oudit, G.Y., Bayraktar, O.A., Seidman, J.G., Seidman, C.E., Noseda, M., Hubner, N., Teichmann, S.A., 2020. Cells of the adult human heart. Nature 588, 466–472. https://doi.org/10.1038/s41586-020-2797-4.

Lu, Z., Jiang, Y.-P., Wu, C.-Y.C., Ballou, L.M., Liu, S., Carpenter, E.S., Rosen, M.R., Cohen, I.S., Lin, R.Z., 2013. Increased persistent sodium current due to decreased PI3K signaling contributes to QT prolongation in the diabetic heart. Diabetes 62, 4257–4265.

Luo, W., Grupp, I.L., Harrer, J., Ponniah, S., Grupp, G., Duffy, J.J., Doetschman, T., Kranias, E.G., 1994. Targeted ablation of the phospholamban gene is associated with markedly enhanced myocardial contractility and loss of beta-agonist stimulation. Circ. Res. 75, 401–409. https://doi.org/10.1161/01.res.75.3.401.

Luo, M., Guan, X., Luczak, E.D., Lang, D., Kutschke, W., Gao, Z., Yang, J., Glynn, P., Sossalla, S., Swaminathan, P.D., Weiss, R.M., Yang, B., Rokita, A.G., Maier, L.S., Efimov, I.R., Hund, T.J., Anderson, M.E., 2013. Diabetes increases mortality after myocardial infarction by oxidizing CaMKII. J. Clin. Invest. 123, 1262–1274. https://doi.org/10.1172/JCI65268.

Marengo, J.J., Hidalgo, C., Bull, R., 1998. Sulfhydryl oxidation modifies the calcium dependence of ryanodine-sensitive calcium channels of excitable cells. Biophys. J. 74, 1263–1277. https://doi.org/10.1016/S0006-3495(98)77840-3.

Marx, S.O., Reiken, S., Hisamatsu, Y., Jayaraman, T., Burkhoff, D., Rosemblit, N., Marks, A.R., 2000. PKA phosphorylation dissociates FKBP12. 6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. Cell 101, 365–376.

Mazzocchi, G., Sommese, L., Palomeque, J., Felice, J.I., Di Carlo, M.N., Fainstein, D., Gonzalez, P., Contreras, P., Skapura, D., McCauley, M., 2016. Phospholamban ablation rescues the enhanced propensity to arrhythmias of mice with CaMKII-constitutive phosphorylation of RyR2 at site S2814. J. Physiol. 594, 3005–3030.

Meo, M., Meste, O., Signore, S., Sorrentino, A., Cannata, A., Zhou, Y., Matsuda, A., Luciani, M., Kannappan, R., Goichberg, P., 2016. Reduction in Kv current enhances

the temporal dispersion of the action potential in diabetic myocytes: insights from a novel repolarization algorithm. J. Am. Heart Assoc. 5, e003078.
Mesubi, O.O., Rokita, A.G., Abrol, N., Wu, Y., Chen, B., Wang, Q., Granger, J.M., Tucker-Bartley, A., Luczak, E.D., Murphy, K.R., Umapathi, P., Banerjee, P.S., Boronina, T.N., Cole, R.N., Maier, L.S., Wehrens, X.H., Pomerantz, J.L., Song, L.-S., Ahima, R.S., Hart, G.W., Zachara, N.E., Anderson, M.E., 2021. Oxidized CaMKII and O-GlcNAcylation cause increased atrial fibrillation in diabetic mice by distinct mechanisms. J. Clin. Invest. 131, e95747 https://doi.org/10.1172/JCI95747.
Miragoli, M., Ceriotti, P., Iafisco, M., Vacchiano, M., Salvarani, N., Alogna, A., Carullo, P., Ramirez-Rodríguez, G.B., Patrício, T., Esposti, L.D., 2018. Inhalation of peptide-loaded nanoparticles improves heart failure. Sci. Transl. Med. 10, eaan6205.
Mishra, S., Gray, C.B., Miyamoto, S., Bers, D.M., Brown, J.H., 2011. Location matters: clarifying the concept of nuclear and cytosolic CaMKII subtypes. Circ. Res. 109, 1354–1362.
Monnerat, G., Alarcón, M.L., Vasconcellos, L.R., Hochman-Mendez, C., Brasil, G., Bassani, R.A., Casis, O., Malan, D., Travassos, L.H., Sepúlveda, M., 2016. Macrophage-dependent IL-1β production induces cardiac arrhythmias in diabetic mice. Nat. Commun. 7, 1–15.
Movahed, M.-R., Hashemzadeh, M., Jamal, M., 2007. Increased prevalence of ventricular fibrillation in patients with type 2 diabetes mellitus. Heart Ves. 22, 251–253.
Mundiña-Weilenmann, C., Vittone, L., Ortale, M., de Cingolani, G.C., Mattiazzi, A., 1996. Immunodetection of phosphorylation sites gives new insights into the mechanisms underlying phospholamban phosphorylation in the intact heart. J. Biol. Chem. 271, 33561–33567. https://doi.org/10.1074/jbc.271.52.33561.
Mundiña-Weilenmann, C., Ferrero, P., Said, M., Vittone, L., Kranias, E.G., Mattiazzi, A., 2005. Role of phosphorylation of Thr17 residue of phospholamban in mechanical recovery during hypercapnic acidosis. Cardiovasc. Res. 66, 114–122. https://doi.org/10.1016/j.cardiores.2004.12.028.
Narayanan, K., Zhang, L., Kim, C., Uy-Evanado, A., Teodorescu, C., Reinier, K., Zheng, Z., Gunson, K., Jui, J., Chugh, S.S., 2015. QRS fragmentation and sudden cardiac death in the obese and overweight. J. Am. Heart Assoc. 4, e001654.
Neef, S., Steffens, A., Pellicena, P., Mustroph, J., Lebek, S., Ort, K.R., Schulman, H., Maier, L.S., 2018. Improvement of cardiomyocyte function by a novel pyrimidine-based CaMKII-inhibitor. J. Mol. Cell. Cardiol. 115, 73–81. https://doi.org/10.1016/j.yjmcc.2017.12.015.
Ninkovic, V.M., Ninkovic, S.M., Miloradovic, V., Stanojevic, D., Babic, M., Giga, V., Dobric, M., Trenell, M.I., Lalic, N., Seferovic, P.M., Jakovljevic, D.G., 2016. Prevalence and risk factors for prolonged QT interval and QT dispersion in patients with type 2 diabetes. Acta Diabetol. 53, 737–744. https://doi.org/10.1007/s00592-016-0864-y.
Nishio, S., Teshima, Y., Takahashi, N., Thuc, L.C., Saito, S., Fukui, A., Kume, O., Fukunaga, N., Hara, M., Nakagawa, M., 2012. Activation of CaMKII as a key regulator of reactive oxygen species production in diabetic rat heart. J. Mol. Cell. Cardiol. 52, 1103–1111.
Nuss, H.B., Marbán, E., Johns, D.C., 1999. Overexpression of a human potassium channel suppresses cardiac hyperexcitability in rabbit ventricular myocytes. J. Clin. Investig. 103, 889–896.
Palomeque, J., Rueda, O.V., Sapia, L., Valverde, C.A., Salas, M., Petroff, M.V., Mattiazzi, A., 2009. Angiotensin II–induced oxidative stress resets the Ca $^{2+}$ dependence of Ca $^{2+}$ –calmodulin protein kinase II and promotes a death pathway conserved across different species. Circ. Res. 105, 1204–1212. https://doi.org/10.1161/CIRCRESAHA.109.204172.
Pereira, L., Ruiz-Hurtado, G., Rueda, A., Mercadier, J.-J., Benitah, J.-P., Gómez, A.M., 2014. Calcium signaling in diabetic cardiomyocytes. Cell Calcium 56, 372–380. https://doi.org/10.1016/j.ceca.2014.08.004.
Pogwizd, S.M., Bers, D.M., 2004. Cellular basis of triggered arrhythmias in heart failure. Trends Cardiovasc. Med. 14, 61–66.
Ponikowski, P., Voors, A., Anker, S., Bueno, H., Cleland, J., Coats, A., Falk, V., González-Juanatey, J., Harjola, V., Jankowska, E., 2016. The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur. Heart J. 37, 2129–2200.
Popescu, I., Yin, G., Velmurugan, S., Erickson, J.R., Despa, F., Despa, S., 2019. Lower sarcoplasmic reticulum Ca2+ threshold for triggering afterdepolarizations in diabetic rat hearts. Heart Rhythm 16, 765–772.
Prosser, B.L., Khairallah, R.J., Ziman, A.P., Ward, C.W., Lederer, W., 2013. X-ROS signaling in the heart and skeletal muscle: stretch-dependent local ROS regulates [Ca2+] i. J. Mol. Cell. Cardiol. 58, 172–181.
Quick, A.P., Wang, Q., Philippen, L.E., Barreto-Torres, G., Chiang, D.Y., Beavers, D., Wang, G., Khalid, M., Reynolds, J.O., Campbell, H.M., Showell, J., McCauley, M.D., Scholten, A., Wehrens, X.H.T., 2017. 'Striated muscle preferentially expressed protein kinase' (SPEG) is essential for cardiac function by regulating junctional membrane complex activity. Circ. Res. 120, 110–119. https://doi.org/10.1161/CIRCRESAHA.116.309977.
Reyes Gaido, O.E., Pavlaki, N., Granger, J.M., Mesubi, O.O., Liu, B., Lin, B.L., Long, A., Walker, D., Mayourian, J., Schole, K.L., Terrillion, C.E., Nkashama, L.J., Hulsurkar, M.M., Dorn, L.E., Ferrero, K.M., Huganir, R.L., Müller, F.U., Wehrens, X. H.T., Liu, J.O., Luczak, E.D., Bezzerides, V.J., Anderson, M.E., 2023. An improved reporter identifies ruxolitinib as a potent and cardioprotective CaMKII inhibitor. Sci. Transl. Med. 15, eabq7839 https://doi.org/10.1126/scitranslmed.abq7839.
Romero-García, T., Landa-Galvan, H.V., Pavón, N., Mercado-Morales, M., Valdivia, H.H., Rueda, A., 2020. Autonomous activation of CaMKII exacerbates diastolic calcium leak during beta-adrenergic stimulation in cardiomyocytes of metabolic syndrome rats. Cell Calcium 91, 102267. https://doi.org/10.1016/j.ceca.2020.102267.
Rusciano, M.R., Sommariva, E., Douin-Echinard, V., Ciccarelli, M., Poggio, P., Maione, A. S., 2019. CaMKII activity in the inflammatory response of cardiac diseases. Int. J. Mol. Sci. 20, 4374. https://doi.org/10.3390/ijms20184374.
Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A.A., Ogurtsova, K., Shaw, J.E., Bright, D., Williams, R., 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res. Clin. Pract. 157, 107843 https://doi.org/10.1016/j.diabres.2019.107843.
Said, M., Vittone, L., Mundiña-Weilenmann, C., Ferrero, P., Kranias, E.G., Mattiazzi, A., 2003. Role of dual-site phospholamban phosphorylation in the stunned heart: insights from phospholamban site-specific mutants. Am. J. Physiol. Heart Circ. Physiol. 285, H1198–H1205. https://doi.org/10.1152/ajpheart.00209.2003.
Said, M., Becerra, R., Palomeque, J., Rinaldi, G., Kaetzel, M., Diaz-Sylvester, P., Copello, J.A., Dedman, J.R., Mundiña-Weilenmann, C., Vittone, L., 2008. Increased intracellular Ca2+ and SR Ca2+ load contribute to arrhythmias after acidosis in rat heart. Role of Ca2+/calmodulin-dependent protein kinase II. Am. J. Physiol. Heart Circ. Physiol. 295, H1669–H1683.
Said, M., Becerra, R., Valverde, C.A., Kaetzel, M.A., Dedman, J.R., Mundiña-Weilenmann, C., Wehrens, X.H., Vittone, L., Mattiazzi, A., 2011. Calcium-calmodulin dependent protein kinase II (CaMKII): a main signal responsible for early reperfusion arrhythmias. J. Mol. Cell. Cardiol. 51, 936–944. https://doi.org/10.1016/j.yjmcc.2011.08.010.
Salas, M.A., Valverde, C.A., Sánchez, G., Said, M., Rodriguez, J.S., Portiansky, E.L., Kaetzel, M.A., Dedman, J.R., Donoso, P., Kranias, E.G., Mattiazzi, A., 2010. The signalling pathway of CaMKII-mediated apoptosis and necrosis in the ischemia/reperfusion injury. J. Mol. Cell. Cardiol. 48, 1298–1306. https://doi.org/10.1016/j.yjmcc.2009.12.015.
Sánchez, G., Araneda, F., Peña, J.P., Finkelstein, J.P., Riquelme, J.A., Montecinos, L., Barrientos, G., Llanos, P., Pedrozo, Z., Said, M., 2018. High-fat-diet-induced obesity produces spontaneous ventricular arrhythmias and increases the activity of ryanodine receptors in mice. Int. J. Mol. Sci. 19, 533.
Santalla, M., Valverde, C.A., Harnichar, E., Lacunza, E., Aguilar-Fuentes, J., Mattiazzi, A., Ferrero, P., 2014. Aging and CaMKII alter intracellular Ca2+ transients and heart rhythm in Drosophila melanogaster. PLoS One 9, e101871. https://doi.org/10.1371/journal.pone.0101871.
Sato, D., Clancy, C.E., Bers, D.M., 2017. Dynamics of sodium current mediated early afterdepolarizations. Heliyon 3, e00388.
Sepúlveda, M., Gonano, L.A., Back, T.G., Chen, S.W., Petroff, M.V., 2013. Role of CaMKII and ROS in rapid pacing-induced apoptosis. J. Mol. Cell. Cardiol. 63, 135–145.
Sepúlveda, M., Gonano, L.A., Viotti, M., Morell, M., Blanco, P., López Alarcón, M., Peroba Ramos, I., Bastos Carvalho, A., Medei, E., Vila Petroff, M., 2017. Calcium/calmodulin protein kinase II-dependent ryanodine receptor phosphorylation mediates cardiac contractile dysfunction associated with sepsis. Crit. Care Med. 45, e399–e408. https://doi.org/10.1097/CCM.0000000000002101.
Shang, Y., Marseglia, A., Fratiglioni, L., Welmer, A., Wang, R., Wang, H., Xu, W., 2019. Natural history of prediabetes in older adults from a population-based longitudinal study. J. Intern. Med. 286, 326–340.
Shao, C.-H., Rozanski, G.J., Patel, K.P., Bidasee, K.R., 2007a. Dyssynchronous (non-uniform) Ca2+ release in myocytes from streptozotocin-induced diabetic rats. J. Mol. Cell. Cardiol. 42, 234–246.
Shao, C.-H., Rozanski, G.J., Patel, K.P., Bidasee, K.R., 2007b. Dyssynchronous (non-uniform) Ca2+ release in myocytes from streptozotocin-induced diabetic rats. J. Mol. Cell. Cardiol. 42, 234–246. https://doi.org/10.1016/j.yjmcc.2006.08.018.
Shao, C.-H., Wehrens, X.H., Wyatt, T.A., Parbhu, S., Rozanski, G.J., Patel, K.P., Bidasee, K.R., 2009. Exercise training during diabetes attenuates cardiac ryanodine receptor dysregulation. J. Appl. Physiol. 106, 1280–1292.
Singh, M.V., Anderson, M.E., 2011. Is CaMKII a link between inflammation and hypertrophy in heart? J. Mol. Med. (Berl.) 89, 537–543. https://doi.org/10.1007/s00109-011-0727-5.
Singh, M.V., Swaminathan, P.D., Luczak, E.D., Kutschke, W., Weiss, R.M., Anderson, M. E., 2012. MyD88 mediated inflammatory signaling leads to CaMKII oxidation, cardiac hypertrophy and death after myocardial infarction. J. Mol. Cell. Cardiol. 52, 1135–1144.
Sommese, L., Valverde, C.A., Blanco, P., Castro, M.C., Rueda, O.V., Kaetzel, M., Dedman, J., Anderson, M.E., Mattiazzi, A., Palomeque, J., 2016. Ryanodine receptor phosphorylation by CaMKII promotes spontaneous Ca(2+) release events in a rodent model of early stage diabetes: the arrhythmogenic substrate. Int. J. Cardiol. 202, 394–406. https://doi.org/10.1016/j.ijcard.2015.09.022.
Souders, C.A., Bowers, S.L., Baudino, T.A., 2009. Cardiac fibroblast: the renaissance cell. Circ. Res. 105, 1164–1176.
Stølen, T.O., Høydal, M.A., Kemi, O.J., Catalucci, D., Ceci, M., Aasum, E., Larsen, T., Rolim, N., Condorelli, G., Smith, G.L., 2009. Interval training normalizes cardiomyocyte function, diastolic Ca2+ control, and SR Ca2+ release synchronicity in a mouse model of diabetic cardiomyopathy. Circ. Res. 105, 527–536.
Sun, J., Yamaguchi, N., Xu, L., Eu, J.P., Stamler, J.S., Meissner, G., 2008. Regulation of the cardiac muscle ryanodine receptor by O2 tension and S-nitrosoglutathione. Biochemistry 47, 13985–13990.
Tada, M., Kirchberger, M.A., Katz, A.M., 1975. Phosphorylation of a 22,000-dalton component of the cardiac sarcoplasmic reticulum by adenosine 3':5'-monophosphate-dependent protein kinase. J. Biol. Chem. 250, 2640–2647.
Tian, C., Hong Shao, C., Moore, C.J., Kutty, S., Walseth, T., DeSouza, C., Bidasee, K.R., 2011. Gain of function of cardiac ryanodine receptor in a rat model of type 1 diabetes. Cardiovasc. Res. 91, 300–309.
Trafford, A., Diaz, M., Sibbring, G., Eisner, D., 2000. Modulation of CICR has no maintained effect on systolic Ca2+: simultaneous measurements of sarcoplasmic

reticulum and sarcolemmal Ca2+ fluxes in rat ventricular myocytes. J. Physiol. 522, 259–270.

Tse, G., Lai, E.T.H., Tse, V., Yeo, J.M., 2016. Molecular and electrophysiological mechanisms underlying cardiac arrhythmogenesis in diabetes mellitus. J. Diabetes Res. 2016, 2848759 https://doi.org/10.1155/2016/2848759.

Valverde, C.A., Mundiña-Weilenmann, C., Reyes, M., Kranias, E.G., Escobar, A.L., Mattiazzi, A., 2006. Phospholamban phosphorylation sites enhance the recovery of intracellular Ca2+ after perfusion arrest in isolated, perfused mouse heart. Cardiovasc. Res. 70, 335–345. https://doi.org/10.1016/j.cardiores.2006.01.018.

Valverde, C.A., Mazzocchi, G., Di Carlo, M.N., Ciocci Pardo, A., Salas, N., Ragone, M.I., Felice, J.I., Cely-Ortiz, A., Consolini, A.E., Portiansky, E., Mosca, S., Kranias, E.G., Wehrens, X.H.T., Mattiazzi, A., 2019. Ablation of phospholamban rescues reperfusion arrhythmias but exacerbates myocardium infarction in hearts with Ca2+/calmodulin kinase II constitutive phosphorylation of ryanodine receptors. Cardiovasc. Res. 115, 556–569. https://doi.org/10.1093/cvr/cvy213.

Van Oort, R.J., McCauley, M.D., Dixit, S.S., Pereira, L., Yang, Y., Respress, J.L., Wang, Q., De Almeida, A.C., Skapura, D.G., Anderson, M.E., 2010. Ryanodine receptor phosphorylation by calcium/calmodulin-dependent protein kinase II promotes life-threatening ventricular arrhythmias in mice with heart failure. Circulation 122, 2669–2679.

Velez Rueda, J.O., Palomeque, J., Mattiazzi, A., 2012. Early apoptosis in different models of cardiac hypertrophy induced by high renin-angiotensin system activity involves CaMKII. J. Appl. Physiol. 112, 2110–2120. https://doi.org/10.1152/japplphysiol.01383.2011.

Vila-Petroff, M., Salas, M.A., Said, M., Valverde, C.A., Sapia, L., Portiansky, E., Hajjar, R. J., Kranias, E.G., Mundiña-Weilenmann, C., Mattiazzi, A., 2007. CaMKII inhibition protects against necrosis and apoptosis in irreversible ischemia-reperfusion injury. Cardiovasc. Res. 73, 689–698. https://doi.org/10.1016/j.cardiores.2006.12.003.

Vila-Petroff, M., Mundiña-Weilenmann, C., Lezcano, N., Snabaitis, A.K., Huergo, M.A., Valverde, C.A., Avkiran, M., Mattiazzi, A., 2010. Ca(2+)/calmodulin-dependent protein kinase II contributes to intracellular pH recovery from acidosis via Na(+)/H(+) exchanger activation. J. Mol. Cell. Cardiol. 49, 106–112. https://doi.org/10.1016/j.yjmcc.2009.12.007.

Watanabe, M., Yokoshiki, H., Mitsuyama, H., Mizukami, K., Ono, T., Tsutsui, H., 2012. Conduction and refractory disorders in the diabetic atrium. Am. J. Physiol. Heart Circ. Physiol. 303, H86–H95. https://doi.org/10.1152/ajpheart.00010.2012.

Wehrens, X.H., Lehnart, S.E., Reiken, S.R., Marks, A.R., 2004. Ca2+/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. Circ. Res. 94, e61–e70.

Wehrens, X.H., Lehnart, S.E., Reiken, S., Vest, J.A., Wronska, A., Marks, A.R., 2006. Ryanodine receptor/calcium release channel PKA phosphorylation: a critical mediator of heart failure progression. Proc. Natl. Acad. Sci. USA 103, 511–518.

Wei, J., Yao, J., Belke, D., Guo, W., Zhong, X., Sun, B., Wang, R., Paul Estillore, J., Vallmitjana, A., Benitez, R., Hove-Madsen, L., Alvarez-Lacalle, E., Echebarria, B., Chen, S.R.W., 2021. Ca2+-CaM dependent inactivation of RyR2 underlies Ca2+ alternans in intact heart. Circ. Res. 128, e63–e83. https://doi.org/10.1161/CIRCRESAHA.120.318429.

Willeford, A., Suetomi, T., Nickle, A., Hoffman, H.M., Miyamoto, S., Brown, J.H., 2018. CaMKIIδ-mediated inflammatory gene expression and inflammasome activation in cardiomyocytes initiate inflammation and induce fibrosis. JCI Insight 3.

World Health Organization, 2022. Obesity. https://www.who.int/health-topics/obesity. (Accessed 13 December 2022).

Xiao, B., Zhong, G., Obayashi, M., Yang, D., Chen, K., Walsh, M.P., Shimoni, Y., Cheng, H., Ter Keurs, H., Chen, S., 2006. Ser-2030, but not Ser-2808, is the major phosphorylation site in cardiac ryanodine receptors responding to protein kinase A activation upon β-adrenergic stimulation in normal and failing hearts. Biochem. J. 396, 7–16.

Xie, Y., Sato, D., Garfinkel, A., Qu, Z., Weiss, J.N., 2010. So little source, so much sink: requirements for afterdepolarizations to propagate in tissue. Biophys. J. 99, 1408–1415.

Yan, J., Bare, D.J., DeSantiago, J., Zhao, W., Mei, Y., Chen, Z., Ginsburg, K., Solaro, R.J., Wolska, B.M., Bers, D.M., Chen, S.R.W., Ai, X., 2021. JNK2, A newly-identified SERCA2 enhancer, augments an arrhythmic [Ca2+]SR leak-load relationship. Circ. Res. 128, 455–470. https://doi.org/10.1161/CIRCRESAHA.120.318409.

Yaras, N., Ugur, M., Ozdemir, S., Gurdal, H., Purali, N., Lacampagne, A., Vassort, G., Turan, B., 2005. Effects of diabetes on ryanodine receptor Ca release channel (RyR2) and Ca2+ homeostasis in rat heart. Diabetes 54, 3082–3088.

Yeh, T.T., Yeap, X.Y., Mullick, A.E., 2020. Downregulation of Ca2+/calmodulin-dependent Protein Kinase Type II Delta in the Heart by Antisense Oligonucleotides Mitigates Development of Post-infarct Ventricular Arrhythmias. In: Am Heart Assoc, p. A438. A438.

Zhang, Y., Xiao, J., Lin, H., Luo, X., Wang, H., Bai, Y., Wang, J., Zhang, H., Yang, B., Wang, Z., 2007. Ionic mechanisms underlying abnormal QT prolongation and the associated arrhythmias in diabetic rabbits: a role of rapid delayed rectifier K+ current. Cell. Physiol. Biochem. 19, 225–238.