Ca^{2+} mishandling in heart failure: potential targets

Short title: New calcium -targets for heart failure

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ABSTRACT

Ca^{2+} mishandling is a common feature in several cardiovascular diseases such as heart failure (HF). In many cases, ~~the~~ impairment of key players ~~of the~~in intracellular Ca^{2+} homeostasis has been ~~determinant in~~identified as the underlying mechanism of cardiac dysfunction and cardiac arrhythmias associated with HF. In ~~the present~~this review, we summarize~~d the main~~ primary ~~and~~ novel findings related to Ca^{2+} mishandling in HF progression. ~~Increasing~~ HF ~~-~~research has increasingly ~~been~~ focused on the identification of new targets and the contribution of their role in ~~the management of~~ Ca^{2+} handling ~~with significance in~~to the progression of the disease. ~~Experimental~~ Recent r~~r~~esearch studies have ~~pointed~~ identified potential targets in ~~out~~ three major emerging ~~fields~~areas implicated in regulation of Ca^{2+} handling~~:,~~ the innate immune system, bone metabolism factors, and post-translational modification~~s~~ of key proteins involved in regulation of Ca^{2+} handling ~~in the regulation of the Ca^{2+} handling~~. Here, we describe~~d~~ their possible contributions to the progression of HF.

1. INTRODUCTION

Heart failure (HF) is a complex syndrome that affects more than 15 million people in Europe.[1–3] HF occurs when the heart is unable to maintain cardiac output at normal filling pressures caused by various aetiologies such as ischaemia, stroke, mechanical stress and pressure overload, genetic diseases, diabetes, or atherosclerosis, among others.[4] HF is commonly associated with mechanical stress-induced cardiac remodelling, neuro-hormonal activation, structural changes, and Ca^{2+} mishandling. ~~Nowadays, o~~Our knowledge of the molecular pathways involved in HF ~~has~~ is continually ~~grown~~growing, ~~rendering~~ revealing HF as a highly complex pathology. Since the eighties, two distinct phenotypes of HF have emerged: HF with reduced ejection fraction (EF) (HFrEF), characterized by systolic dysfunction, and HF with preserved EF (HFpEF), with diastolic dysfunction. Recently, Kilfoil PJ ~~-~~et al.~~,~~ ~~have~~ described whether different~~-~~ regulation ~~in~~ of Ca^{2+} handling is shown in HFrEF or HFpEF.[5] ~~This~~ Our review focuses on the cardiomyocyte intracellular Ca^{2+} dependent mechanisms involved in early, mild or end stages of HFrEF.

HFrEF is characterized by ~~a~~ depressed cardiomyocyte contractile function, which ~~of cardiomyocytes~~ lead~~s~~ing to reduced left ventricular contraction during systole. D~~T~~he ~~d~~efective cardiac contractility is associated with an impaired excitation-contraction (EC) coupling, a mechanism that converts electrical stimuli from ~~the~~ pacemaker cells into contraction ~~via~~

through a massive Ca^{2+} release from the sarcoplasmic reticulum (SR).[6] The most common changes ~~of~~ in EC coupling associated with HF are: a) reduced systolic SR-Ca^{2+} release through type 2 ryanodine receptors ~~type 2~~ (RyR2s), b) decreased reuptake of Ca^{2+} into the SR by the Sarcoplasmic/Endoplasmic Reticulum Ca^{2+} ATPase 2a (SERCA2a pump), c) increased Ca^{2+} extrusion through sodium-calcium exchanger (NCX), and d) increased diastolic SR Ca^{2+} leak. All of these alterations contribute to reducing SR Ca^{2+} load, limiting the amount of SR Ca^{2+} needed to produce optimal cardiomyocyte contraction~~s~~. Moreover, several studies have reported a reduction in the maximal force-generating capacity of the myofilaments in HF, as well as~~,~~ biochemical alterations of the contractile apparatus, including suppression of α-myosin heavy chain expression,[7] switching of troponin T isoforms,[8] and decreased basal cyclic adenosine-monophosphate (cAMP)-dependent phosphorylation.[9] ~~More~~ Additional elements are likely involved in the control of cardiac intracellular Ca^{2+} handling, such as transient receptor potential (TRP) channels. ~~The fine~~Exquisite orchestration of all ~~these~~ elements ~~results~~ is required for ~~in an~~ adequate EC-coupling and cell contraction.

This review ~~aims~~ seeks to provide an overview of the main processes underlying changes observed in EC coupling during HF. We have also ~~compelled~~ compiled interesting data relat~~inged~~ to promising new targets in Ca^{2+} handling management, uncovering emerging research areas ~~such as~~ including new mediators of the immune system, bone metabolism factors, and post-translational modifications of key proteins involved in Ca^{2+} handling regulation.

2. BASIS OF EXCITATION-CONTRACTION COUPLING

~~In the heart,~~ EC coupling in the heart relies on the Ca^{2+}-induced Ca^{2+}-release (CICR) mechanism. ~~Following~~ E~~e~~lectrical stimulation from the sinoatrial node ~~and~~ travels through the conduction system during the plateau phase of the cardiac action potential, triggering an inward Ca^{2+}current (I_{CaL}) ~~from~~ through ~~the~~ voltage-dependent L-type Ca^{2+} channels (LTCCs, localized in the T-tubules). I_{CaL}, in turn, stimulates the opening of ~~Ca^{2+} channels/ryanodine receptors type 2 (~~RyR2s~~)~~ found in the junctional SR (jSR)~~.~~ The RyR2s~~that mediate the release of a large quantity of Ca^{2+} from luminal SR into the cytoplasm, increasing free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Ca^{2+} binds to troponin C, ~~allowing~~ enabling cardiac contraction. After cardiomyocytes contract, the $[Ca^{2+}]_i$ returns to diastolic levels, which lead~~sing~~ to cardiomyocyte~~s~~ relaxation. There are two principal mechanisms by which Ca^{2+} is removed from the cytoplasm: a) Ca^{2+} is pumped back to the SR by the SERCA2a pump, and b) Ca^{2+} is

extruded via the NCX. Additional minor mechanisms contribute to Ca^{2+} removal, including the plasmalemma Ca^{2+}-ATPase and the mitochondrial Ca^{2+} uniporter (Panel I, Figure 1).[10]

2.1 Key actors in ~~the~~ EC-Coupling

2.1.1 L-Type Calcium channel

The LTCC is a macromolecular protein complex comprised of pore-forming Cav1.2 (α subunit) and auxiliary subunits that modulate channel function.[11,12] These channels are ~~mainly~~ primarily found on the transverse (T)-tubules of cardiomyocytes and are activated by depolarization of the sarcolemma.[13,14] LTCCs ~~have~~ play a central role during the plateau phase of the action potential by allowing an inward *ICaL* for cardiac EC coupling,[13] –intracellular signalling pathways,[15] and gene regulation.[16] T-Tubular LTCCs and RyR2s from the SR membrane associate closely to form a dyad microdomain in ventricular cardiomyocytes. Their proximity is essential to developing ~~an adequate~~ sufficient CICR ~~process~~. ~~Application~~ Studies of healthy rat and human ventricular myocytes using ~~of~~ super-resolution scanning patch-clamp, along with confocal and fluorescence microscopy techniques ~~to healthy rat and human ventricular myocytes~~, ~~indicated~~ demonstrated that the probability of finding LTCCs on the sarcolemmal surface was 3- to 4- fold less than in T-tubules,[17] which is consistent with previous reports ~~supporting~~ reporting that the majority of ventricular LTCCs are found on T-tubules.[18] Evidence suggests that some regulatory proteins such as junctophilin-2 (JPH2), ~~or~~ protein bridging integrator 1, or amphiphysin-2 (BIN1) are crucial for the maintenance of dyad microdomain integrity.[19] JPH2 is essential for T-tubologenesis during postnatal development of the heart~~,~~[20] ~~and~~ because it promotes T-tubule~~s~~ structural stability, ~~allowing~~ enabling connections between T-tubule invaginations to functional SR organelles in order to maintain efficient ECC.[21,22] BIN1 is reported to play a key role in LTTC trafficking to the T-tubules[23] and in the process of folding the T-tubules' inner membrane to limit ion diffusion.[24]

The major functional regulation of LTCCs occurs at its cytosolic C-terminal region, which contain~~s~~ing various phosphorylation residues involved in fast regulatory responses,[25] as well as the IQ motif, a specific interaction domain for calmodulin.[26] Calmodulin binding to the IQ motif modulates LTCC function by inducing Ca^{2+}-dependent inactivation or Ca^{2+}-dependent facilitation.[26–28]

2.1.2 The type 2 ryanodine receptors

The RyR2s are high-conductance intracellular Ca^{2+} channels that mediate the release of Ca^{2+} from the SR. Among mammalians RyR isoforms, type 2 represents the most abundant subtype in cardiomyocytes. The RyR2 is the largest ion channel currently known, with a molecular mass exceeding 2.2 MDa. A single RyR2 channel is assembled as a homotetramer, in which each subunit contains 4,968 aa's amino acid residues. Images from optical super-resolution microscopy have demonstrated that cardiac RyR2s are organized in functional groups or clusters within the Ca^{2+} release units (CRU's), which , the latter are constituted comprised of of four proteins: LTCCs, RyR2s, junctin, and triadin, located in the jSR.[29,30]. RyR2 contains two functional domains: (1) the central rim formed by the N-terminal, central and pore-forming domains; and (2) the external region containing the handle, P1, and P2 domains. Of main primary importance is the phosphorylation domain in P2 that contains many targets for multiple kinases (mainly of which Ser^{2808}, Ser^{2814}, and Ser^{2030} are most important in regulating Ca^{2+} homeostasis) for multiple kinases.[30–33] The RyR2 also forms a complex with two major protein kinases, the cAMP-dependent protein kinase A (PKA) and the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), as well as three protein phosphatases (PP1, PP2, and PP2B). It is thus clear that indicating the importance of RyR2 phosphorylation plays a crucially important role in Ca^{2+} regulation.[34]

RyR2s is activated when $[Ca^{2+}]_i$ reaches a certain level into in the dyad or when the SR-free Ca^{2+} ($[Ca^{2+}]_{SR}$) is over theexceeds physiological levels.[35] RyR2s regulation relies on several mechanisms, including a) direct Ca^{2+} interactions, both at the cytosolic and luminal sides; b) accessory cytoplasmic regulatory proteins, such as 12.6-KDa FK506-binding protein (FKBP12.6), sorcin[36] and JPH2;[37] and c) SR luminal proteins, for instance, calsequestrin, triadin, and junctin.[38]

SA suitable inactivation of RyR2s is critical to minimize inappropriate SR Ca^{2+} release events between heartbeats.[10] Several mechanisms are participateing in the termination of Ca^{2+} release via RyR2: (1) Ca^{2+}-dependent inactivation/adaptation of RyR2s by cytoplasmic and luminal-SR proteins,; (2) spontaneous decay of RyR2s activity due to stochastic attrition, and (3) depletion of SR-Ca^{2+} stores, which that induces the RyR2 inactivation.[39] There are different forms of diastolic Ca^{2+} release: Ca^{2+} quarks, Ca^{2+} sparks, Ca^{2+} waves, or and spontaneous Ca^{2+} transients.[40] Ca^{2+} sparks have a physiological role in maintaining the balance of SR-Ca^{2+} stores balanced between systole and diastole.

2.1.3 Sarco/endoplasmic reticulum Ca^{2+} ATPase 2a

SERCA2a is the predominant cardiac isoform of SERCA, which ~~and~~ controls cytosolic Ca^{2+} removal rate and SR refilling (SR Ca^{2+} load) in cardiomyocytes. SERCA2 is distributed in the SR (longitudinally and transversely) and near the T-tubules throughout cardiomyocytes.[41] To transfer Ca^{2+} ions into the SR, SERCA2a uses two specialized domains: E1 and E2. During cardiomyocyte relaxation, Ca^{2+} binds to E1~~,~~ after ATP binding[42]; ~~and the~~ SERCA2a then pumps Ca^{2+} into the SR lumen to restore $[Ca^{2+}]_{SR}$ steady-state. Although SERCA2a interacts with a wide array of proteins (including HRC, PP1~~,~~ calreticulin, S100A, and sarcolipin), phospholamban (PLN) is the most important regulator of its activity.[43] ~~At~~ In ~~the~~ its unphosphorylated state, PLN inhibits SERCA2a activity by lowering the Ca^{2+} affinity of the pump. PLN has two relevant phosphorylation sites: Ser^{16}, a target of cAMP and cGMP-dependent protein kinases~~,~~ such as PKA and protein kinase C (PKC)~~,~~ and Thr^{17} by CaMKII.[44,45] P~~LN p~~hosphorylation of PLN at either of these residues results in the formation of pentameric complexes, relieving PLN inhibition on SERCA2a and ~~and~~ increasing its pumping rate, thereby enhancing SR Ca^{2+} uptake. ~~Specifically, Thr^{17} phosphorylation by CaMKII increases SR Ca^{2+} uptake; while phosphorylation of Ser^{16} also enhances SERCA2a activity and SR Ca^{2+} uptake.~~[46] Finally, SERCA2a is highly sensitive to cytosolic metabolic changes, including the ATP/ADP ratio, pH, and redox potential.[47,48]

2.1.4 Sodium Calcium exchange and Voltage-gated Na^{2+} channel

The NCX is the main route for Ca^{2+} extrusion from the cardiomyocyte. NCX and others participate in cardiomyocyte relaxation by restoring cytosolic Ca^{2+} levels. In the forward mode, NCX exchanges one Ca^{2+} for three Na^+ ($I_{Na/Ca}$);[49] ~~while~~ in the reverse mode, NCX produces Ca^{2+} influx. The driving force determining NCX direction and function is the electrochemical gradient (~~Ca^{2+} and Na^+~~ membrane potential and Ca^{2+} and Na^+ transmembrane gradient).[50] NCX is regulated ~~by~~ (1) by the small inhibitory protein phospholemman and ~~it is~~(2) through phosphorylation~~ed~~ by PKA and PKC, both of which induce~~ing~~ its inhibition.[51] During PKA and PKC phosphorylation, phospholemman increases cardiomyocytes' contractility by inhibiting the forward mode of NCX ~~forward mode~~ and increasing $[Ca^{2+}]_i$.

The sodium (Na^+) current (I_{Na}) in ventricular cardiomyocytes is composed ~~by~~ of a peak (I_{Na-P}), responsible for the initial upstroke of the AP~~,~~ and a late current (I_{Na-L}) ~~which~~ that contributes to the formation of the AP plateau. I_{Na} is produced by the cardiac isoform of the voltage-gated Na^+ channel (Nav1.5, Uniprot entry Q14524) operating in special gating modes.[52] ~~The Na^+~~This channel is a hetero-multimeric protein composed of a pore-forming α subunit of 2,016

aa~~'s~~ residues~~,~~ encoded by the ~~-~~*SCN5A* ~~-~~gene, and auxiliary β subunits. The α subunit consists of four homologous domains (DI–DIV). Each domain contains 6 transmembrane segments (S1–S6), of which the S4 segment functions as a voltage sensor and the S5 and S6 regions form the pore with an ~~-~~intermembrane P-loop.[53] More than 400 mutations have been identified in the SCN5A gene and are associated with an increasingly wide range of congenital arrhythmias including long QT syndrome 3 and Brugada syndrome 1.[53] ~~Also~~Moreover, the participation of $I_{Na\text{-}L}$ in the pathophysiology of HF has been extensively studied.~~pathophysiological participation of $I_{Na\text{-}L}$ in HF has been studied long ago[52], and compelling reviews have been written about it.~~[54,55]

2.1.5 Transient receptor potential channels

The transient receptor potential (TRP) channels are cation channels that contribute to ~~the~~ Ca^{2+} influx evoked by a wide spectrum of chemical and physical stimuli in cardiac cells.[56] Since their first discovery, several TRP isoforms have been identified and are~~,~~ grouped into six major subgroups based on their specific function and sequence analogies~~, have been identified~~: (1) TRPC (the canonical channel), (2) TRPV (the vanilloid-related channel), (3) TRPM (the melastatin-related channel), (4) TRPA (the ankyrin-related channel), (5) TRPP (polycystin-related channel), and (6) TRPML (the mucolipin-related channel~~) (see for review~~).[57,58] The expression of TRP isoforms in the heart was examined in isolated cardiomyocytes, ~~in~~ cardiac cell lines, and in heart tissue, as reviewed elsewhere.[56,59]

TRP channels can be activated by vasoactive agonists (e.g., endothelin-1, thrombin, ATP, angiotensin-II, or bradykinin)~~,~~; ~~by~~ extracellular ions (e.g., H^+, Ca^{2+}, and Mg^{2+})~~,~~ or intracellular second messengers (e.g., diacylglycerol (DAG), phosphoinositide-4,5-bisphosphate (PIP_2)), or by temperature and mechanical stretch, as reviewed elsewhere.[60–62] Interestingly, functional TRP channels can be formed by homomeric and heteromeric oligomerization of TRPC, TRPM and TRPV subunits.[63–65]

Considerable evidence suggest~~sed~~ that TRP channels, especially TRPC isoforms, play a role in ~~the~~ store operated Ca^{2+} entry (SOCE) in cardiac myocytes.[66] SOCE is a Ca^{2+} entry pathway driven mainly by Orai1, the pore-forming sub-unit of the channel~~,~~[67] ~~which~~ Orai 1 is activated by intercellular Ca^{2+} store~~s~~ depletion~~,~~, which is ~~-~~detected by STIM1 (Stromal Interaction Molecule 1), a Ca^{2+} sensor located in the sarcoplasmic reticulum ~~called STIM1 (Stromal Interaction Molecule 1)~~.[68]

To examine ~~the~~ intracellular Ca^{2+} handling in cardiac myocytes, cells are routinely either treated with (1) receptor agonists ~~that~~s evok~~eing~~ IP_3-dependent Ca^{2+} release from the intracellular store or ~~by~~ (2) drugs that deplet~~eing~~ the store, which activates ~~the~~ SOCE. The participation of TRPC1, C3, C4, C5 and C6 in SOCE has been examined in adult rodent cardiac myocytes, in cardiac cell line and in neonatal rat ventricle myocytes, using RNA silencing, neutralizing antibodies, or dominant-negative transgenic mice~~transgenic mice expressing that are dominant-negative of for these proteins~~.[69–73]

3. Ca^{2+} MISHANDLING IN HEART FAILURE

3.1 EC uncoupling in heart failure

HF is commonly associated with a subcellular dys-synchrony related to ~~the~~ detrimental structural and functional ~~detrimental~~ remodeling of the cardiomyocyte.[6,47,74] Indeed, the loss of the dyadic structure observed in HF, together with a reduction in the overall T-tubule~~s~~ density and the consequent loss of tight coupling between LTCCs and RyR2s, results in desynchronized CICR and reduced Ca^{2+} transient amplitude. This, in turn, leads ~~ing~~ to contractile dysfunction, as observed in rats with experimental ~~heart failure~~HF induced by left coronary artery ligation.[75] As part of the dyad, LTCCs on the T-tubules and RyR2s on the SR are closely associated to control the control over CICR Structural alteration of T-tubules has been repeatedly observed in animal and human failing hearts associated with ischemic heart disease, idiopathic dilated cardiomyopathy, and hypertrophic obstructive cardiomyopathy.[18,76] It has been reported recently that disruption of membrane structure associated with HF (decrease in regularity and internal density of T-tubules), led to the redistribution of LTCCs from T-tubules to the sarcolemmal surface (extradyadic space). Interestingly, these redistributed LTCCs show a significant increase in open probability (P_o), which could be linked to ~~a~~ higher phosphorylation of the channel[12,77] and might ~~help to understand~~clarify ~~why~~ the widely reported, perplexing observation that whole-cell I_{CaL} density ~~has been widely reported to not be~~is not altered in failing cardiomyocytes.[78,79] Moreover, it has been postulated that the delocalization-induced increase~~d~~ in channel activity can be associated with an enhancement of net inward currents during the plateau phase of the AP (window I_{CaL} ~~but also~~and late I_{Na}) that can contribute to the development of early after depolarizations ~~development~~ and ventricular arrhythmogenesis in HF.

T-tubule adaptor proteins JPH2 and BIN-1 play an important role in T-tubule~~s~~ remodeling and dyad uncoupling during heart failure progression. As previously explained, JPH2 promotes T-

tubule structural stability~~,~~ and connects T-tubules to the junctional SR.[21,80,81] Interestingly, JPH2 is downregulated in the failing heart of patients and mice with hypertrophic cardiomyopathy~~;~~.[82,83] ~~suggesting that may be~~this downregulation could represent an early molecular event preceeding pathological remodeling. Moreover, cardiac specific JPH2 knockdown in adult mice result~~sed~~ in HF and increased mortality,[21] whereas JPH2 gene therapy prevent~~sed~~ loss of T-tubules and suppressed abnormal SR Ca^{2+} leak associated with contractile failure following transverse aortic constriction (TAC) in mice.[84] ~~Besides~~Moreover, a significant reduction in the expression of the ~~-~~scaffold protein BIN-1 has been reported in failing human hearts [85] and in experimental models of HF induced by overload or ischemia.[86,87] Decreased BIN1 levels promote T-tubule~~s~~ loss~~t~~[86] and T-tubule~~s~~ folding reduction,[24] impairing dyad formation, calcium transient regulation, and cardiac contractility.[86,88]

Recent studies have shown that cardiac BIN1 replacement therapy can improve myocardial function and Ca^{2+} handling ~~-~~in mice with pre-existing HF~~-~~ (Li et al. 2020; Liu et al. 2020).[89,90] Also, BIN1 can be detected in plasma~~:~~ ~~and~~ several studies have proposed ~~BIN1~~ use of the protein as a potential biomarker for pathological cardiomyocyte remodeling in patients with HFpEF.[85,91]

3.2 Reduced Sarcoplasmic reticulum Ca^{2+} load in heart failure

HF is usually associated with depressed SR Ca^{2+} load, mostly due to ~~an~~ impairment ~~in~~ of ~~the~~ SR Ca^{2+} re-uptake by SERCA2a; ~~and~~ in some cases, HF is associated with increased diastolic RyR2 leak. Classically, the majority of studies have demonstrated decreased SERCA2a activity in patients with HF. In many cases, SR-Ca^{2+} load in HF is reduced, in part, due to the down-expression of SERCA2a, which ~~-~~compromise~~sing~~ SR Ca^{2+} reuptake, as observed in ischemic HF patients and in post-myocardial infarction animal models.[92–94] ~~As~~ Because SERCA2a is unable to resequester~~uptake~~ ~~all the~~ sufficient Ca^{2+} ~~for~~ to enable relaxation ~~to occur~~, NCX expression levels are increased as a compensatory mechanism to extrude the excess intracellular Ca^{2+} necessary ~~excess~~ to maintain $[Ca^{2+}]_i$.[95,96] Both diminished SERCA2a function and augmented NCX activity tend to reduce SR Ca^{2+} content, limiting SR Ca^{2+} release through RyR2s and decreasing both systolic Ca^{2+} release and cardiomyocyte contractility, as described in a HF rabbit model induced by aortic insufficiency.[10]

SERCA2a gene therapy has been under evaluation in clinical trials for new HF treatments.[97–101] Some authors have ~~described~~ observed that ~~the~~ introduction of SERCA2a into isolated cardiomyocytes from HFrEF patients and in experimental models results in the improvement of myocardial contractility.[102,103] However, discrepancies regarding the ~~beneficial~~ benefits ~~role~~

of targeting SERCA2a in the clinical practice ~~arose~~have arisen.[101,104,105] Gene therapy targeting SERCA2a was tested in the Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) trials. In the CUPID1 trial, intracoronary adeno-associated virus type 1 (AAV1)/SERCA2 or placebo was administrated to 39 patients with advanced HF: ~~and~~ a reduction in clinical events and hospitalization duration ~~were~~ was observed.[104] However, these results were not confirmed in the CUPID2 trial.[101] CUPID2 was a phase 2b multinational, double-blind, placebo-controlled trial that included 250 patients with HFrEF: the trial failed to ~~that did not show~~detect any evidence of improved outcomes at the evaluated dose of AAV1/SERCA2.[101] The failure effect ~~can~~ might be explained by ~~a possible~~ low efficiency of gene transduction or, ~~maybe~~ perhaps, by post-translational regulatory factors of SERCA2 in human HF. ~~In fact,~~C~~regarding~~ompounding the discouraging results of the CUPID2 trial, other clinical trials stopped recruiting patients ~~as~~ when the SERCA-LVAD trial, which assessed the feasibility and security of AVV1/SERCA2a delivery in human hearts, ~~finally found~~concluded ~~that~~ after patients follow-up that ~~the~~ total transgene DNA levels were very low ~~with~~ and produced no functional benefit.[106] Post-translational modifications of SERCA2 result in alteration of its activity and stability, as observed in patients with non-ischemic cardiomyopathy.[107] SERCA2a undergoes redox modifications that often promote~~ing~~ SERCA2a inhibition and SR-Ca^{2+} depletion, as seen with SERCA sulfonylation at cysteine-674 (Cys-674) and nitration at tyrosine- 294/295 ($Tyr^{294/295}$), which block~~ing~~ ATPase function and ~~participating~~elicit ~~in~~ changes in SR-Ca^{2+} uptake, ~~and~~ inducing cardiac dysfunction in senescent mice and rat hearts.[108,109] More recently, ~~in~~ a study investigating a mouse model of propionic acidemia ~~that~~ with ~~harbour~~ systolic impairment observed that ~~,~~ oxidized methionine-361 (Met^{361}) dethiomethylation of 207, 220, 239, 452 and 622 ~~have described, these changes were~~was closely associated with ~~a~~ depressed SR Ca^{2+} uptake by SERCA2a, thus compromising SR-Ca^{2+} load and cell contractility in ~~this~~ these mice.[110] Importantly, the small ubiquitin-related modification 1 (SUMO1) of SERCA2 (SUMOylation) has been shown to decrease significantly in human HF; conversely, ~~, while~~ SUMO1 restitution by adeno-associated-virus-mediated gene delivery maintained SERCA2a protein levels and significantly improved cardiac function in mice with HF induced by TAC.[95] ~~So~~Thus, increasing~~ed~~ ~~pieces of information~~evidence relating~~ed~~ to posttranslational changes in SERCA2a ~~appear~~ is coalescing into~~as~~ an emerging field of research into the healthy and failing heart and will hopefully help ~~to~~ uncover new targets ~~to~~ for improving~~e~~ cardiac contractility in HF.

As previously mentioned, PLN is the main regulator of SERCA activity. During HF, phosphorylation levels of PLN are decreased either at Ser^{16},[111,112] Thr^{17},[113] or both,[114,115]

resulting in SERCA2a inhibition and depletion of SR Ca^{2+} content. This decrease in PLN phosphorylation can result from an increase in phosphatase PP1 activity, as observed in patients with HF due to idiopathic dilated cardiomyopathy,[111] or a diminution of Thr^{17} phosphorylation due to elevated PP2B (calcineurin) activity.[116] Interestingly, cardiac-specific overexpression of PLN impairs Ca^{2+} handling through inhibition of SR Ca^{2+} uptake, leading to reduced SR Ca^{2+} load and contractile dysfunction.[117] In contrast, PLN down-expression results in enhanced SR Ca^{2+} uptake and contractile function due to a higher affinity of SERCA2a for Ca^{2+}.[118] Identification of several mutations in the human PLN gene of HF patients with arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy and peripartum cardiomyopathy has highlighted a prominent role of PLN in EC coupling.[119] Notably, a heterozygous deletion of Arg^{14} (R14del) of the PLN protein has been identified mostly in the Netherlands: carriers were at high risk for developing ventricular arrhythmias and HF, and were diagnosed with arrhythmogenic cardiomyopathy.[120] Arginine 14 is involved in the PKA phosphorylation site at Ser 16; thus, a mutation in Arg14 of PLN could be related to partial disruption of the stability of the PLN pentamer, leading to augmented PLN monomer concentration and, consequently, to SERCA2a inhibition.[121]

To elucidate the molecular mechanism underlying the pathogenesis of the R14del-PLN mutation, patient-specific iPSC-derived cardiomyocytes (iPSC-CMs) from a patient carrying the mutation were generated. The iPSC-CMs exhibited progressive Ca^{2+} handling impairment and an arrhythmic profile, along with abnormal cytoplasmic distribution of PLN protein, which correlates with the fatal arrhythmias and abnormal PLN cellular aggregation observed in R14del patients.[122] In addition, heterozygous PLN-R14del mice developed cardiac dysfunction, increased myocardial fibrosis and PLN protein aggregation after 18 months. Moreover, standard HF therapy with beta-blockers could not reverse disease progression in heterozygous PLN-R14 del mice.[123] All these results provide key insights into the role of PLN in HF as a key regulator of SERCA2a activity, controlling SR Ca^{2+} load and the cardiac contraction-relaxation cycle.

3.3 Increased diastolic Ca^{2+} leak as a pro-arrhythmogenic mechanism associated with HF

Diastolic RyR2 activity increases significantly in HF, resulting in increased diastolic SR Ca^{2+} leak. This leak, in turn,

~~corresponding to~~triggers spontaneous RyR2~~s~~ openings ~~in~~ during the refractory period, diminishing ~~the~~ SR-Ca^{2+} load (Panel II, Figure 1).

Several studies have reported increased diastolic Ca^{2+} leak, measured as Ca^{2+} sparks or Ca^{2+} waves in animal models ~~experimental~~ and human failing hearts.[47,50,124–126] Diastolic Ca^{2+} leak can be related to several factors, ~~such as~~including ~~the higher~~increased ~~activity of~~ modulatory protein~~s~~ activity or elevated posttranslational modification~~s~~: for instance, phosphorylation or oxidation. Also, the ~~higher~~ elevated SR Ca^{2+} leak in HF may increase the likelihood of triggered arrhythmogenic events propagating as Ca^{2+} waves, which activate a transient inward current via NCX, ~~that~~ giving~~es~~ rise to arrhythmogenic delayed after-depolarizations (DADs), as described in overload ~~or~~ and ischemia HF animal models.[126–129] HF can also be linked to enhanced or "hyper"-phosphorylation and redox modification of RyR2s: ~~that~~ in the majority of cases, these modifications induced increased diastolic Ca^{2+} leak.[130–134]

As previously mentioned, NCX is a key regulator of intracellular Ca^{2+} content.[135] NCX upregulation is a common feature of both human and animal HF.[136,137] As such, changes often occur simultaneously with SERCA2a downregulation, so that a marked increase in NCX/SERCA2a ratio is commonly reported~~,~~ ~~and it~~ this altered ratio has been implicated in both cardiac dysfunction and arrhythmogenesis.[138] Chronic up-regulation of NCX results in maladaptive cardiac remodeling ~~since~~ due to the fact that NCX extrudes $[Ca^{2+}]_i$ rather than ~~does not~~ restoring SR Ca^{2+} stores.[139] ~~As~~ Further, because NCX is an electrogenic ion exchanger, ~~the~~ more Ca^{2+} ~~is~~ extruded from the cardiomyocyte means that~~,~~ ~~the~~ more Na^+ enters. ~~Under~~ In pathological conditions, this added Na^+ influx can depolarize the cardiomyocyte membrane, generating new action potentials that lead to pro-arrhythmogenic events, as described in a rabbit model of HF induced by combined aortic insufficiency and stenosis.[128,140] Increased diastolic SR Ca^{2+} release is related to augmented NCX activity in HF, leading to a ~~greater~~ larger Na^+ inward transient current, which will produce larger depolarizations and promote~~ing~~ DADs.

~~Among the actors involved in the generation of diastolic HF-associated Ca^{2+} leak, the hyper-phosphorylation of RyR2 have gained much interest.~~

3.3.1 Post-translational modifications of RyR2 in heart failure

RyR2 hyper-phosphorylation has generated significant interest as a putative key actor ~~Among the actors involved in the generation of diastolic HF-associated Ca^{2+} leak, the hyper-phosphorylation of RyR2 have gained much interest.~~

RyR2 phosphorylation. During the progression of HF, biochemical defects arise in the beta-adrenergic receptor (β-AR) signalling pathway. Maladaptive c~~C~~hronic β-AR activation during HF ~~is maladaptive and~~ results in Ca^{2+} handling dysregulation, ~~mainly~~ primarly by inducing posttranslational changes in RyR2, ~~that~~ which, together with cellular effects, promotes ~~the~~ progression to myocardial failure,[141–144] disruption of cardiac contractility, ~~promote~~ arrhythmogenic events, and cardiac dysfunction (Panel II, Figure 1).[145]

According to Marks'~~'s~~ hypothesis, ~~in HF,~~ RyR2 hyper-phosphorylation in HF causes FKPB12.6 dissociation and RyR2 channel "leakiness," ~~,~~as reported in RyR2-S2808A post-MI mice ~~that underwent myocardial infarction~~ and in calstabin2-deficient mice.[146–148] Specific RyR2 sites are hyper-phosphorylated by PKA (Ser^{2808} and Ser^{2830}~~,~~ in mice) and by CaMKII (Ser^{2808} and Ser^{2814}~~,~~ in mice) during HF. ~~A~~ The general ~~agreement~~ consensus is that CaMKII phosphorylation of RyR2 opens the channel, favouring Ca^{2+} leak and DADs,[127,149,158,159,150–157] although some authors describe PKA phosphorylation of RyR2s as the main mechanism of abnormal diastolic Ca^{2+} leak.[160–165] Nevertheless, several studies have also shown that PKA-mediated RyR2 phosphorylation has little or no functional relevance for RyR2-mediated Ca^{2+} leak when SR Ca^{2+} levels remain constant;[166] ~~while~~ other research groups have ~~reported~~ arrived at different conclusions.[167,168] Still, whether Ser^{2808}, Ser^{2814}~~,~~ or Ser^{2830} are hyper-phosphorylated in HF remains controversial. Several studies have attempted to elucidate the mechanism responsible for diastolic Ca^{2+} ~~-~~leak, showing that Ser^{2808} only, Ser^{2814} only, neither residue, or both Ser^{2808} and Ser^{2814} are hyper-phosphorylated in failing heart tissue from patients with ischemic cardiomyopathy and HF mice models.[130,134,148] In addition, Ser^{2808} hyper-phosphorylation has been shown to enhance both the open state of the channel and diastolic Ca^{2+} ~~-~~leak in animal models of HF, depleting SR Ca^{2+} ~~-~~load and impairing EC coupling.[132,151,169] Indeed, high-resolution ~~-~~RyR2 ~~-~~cryo-EM ~~-~~structures showed that $Ser^{2808,2814}$ phosphorylations lead to a conformation that promotes~~facilitates~~ ~~its~~ the channel's open state, enhancing diastolic Ca^{2+} leak.[170] A recent and very elegant work from Van Petegem´s group has shown that prior phosphorylation of Ser^{2814}, a target site of CaMKII, induces an alpha helix formation in the phosphorylation domain, facilitating PKA-RyR2 interaction. Hence, ~~the~~ RyR2 phosphorylation sites are not independent;[171] ~~and~~ more evidence favors ~~a~~ synergistic activity between both kinases (CaMKII and PKA) as an underlying mechanism in the diastolic Ca^{2+} leak and arrhythmogenic activity ~~in~~ associated with HF.

On the other hand, one study has reported that Ser^{2030} ~~-~~phosphorylation ~~-~~remains unaltered in a HF rat model with congestive myocardial infarction.[172] ~~These~~ Clearly, these conflicting results

differences needmust to be clarified with new approaches given the importance of the roles played by that PKA and CaMKII play important roles in the regulation of cardiac EC coupling in the heart.

During HF, there is also associated with an increase in the phosphatase expression levels;, but however, less activity of cellular phosphatase is associated with RyR2s.[173] Indeed, reduced PP1 and PP2A activity in the RyR2 macromolecular complex have been shown to modify RyR2 phosphorylation levels in rabbits with HF induced by aortic insufficiency followed by aortic constriction.[151] Pharmacological inhibition of PP2A results in hyper-phosphorylation of the RyR2 at site Ser^{2814}, promoting diastolic Ca^{2+} leak.[174] In contrast, unchanged levels of PP1A catalytic subunits have also been reported in a canine HF model induced by right ventricular tachypacing.[175] Other studies have described reduced protein levels of PP1 inhibitor I-1 in human failing cardiomyocytes from patients with dilated and ischemic cardiomyopathy,[176] with restored contractility achieved rescued by through genetic overexpression of this inhibitorI-1.

PDEs have also been identified in the RyR2 complex as the main route to lower cAMP and cGMP levels inside the cells. Modification of both PDE expression and activity has been observed in a canine HF model induced by rapid cardiac pacing.[132] There are eleven PDE families with different primary structures, catalytic properties, and regulatory mechanisms. On one hand, PDEs 2/3/4 regulate the activity of PKA through cAMP hydrolysis,[177] modulating β-adrenergic response, PKA-dependent RyR2 phosphorylation, and cardiomyocyte contractility.[178] Specifically, PDE4 deficiency has been shown to induce arrhythmogenesis in animal models of HF by PKA hyper-phosphorylation of RyR2.[179] On the other hand, PDE1/2/5/9 regulate cGMP levels and are overexpressed in HF, leading to maladaptive effects.[180] Therefore, the principal role of phosphatases and PDEs in HF remains controversial, although emerging evidence suggests a disturbed balance between kinases, phosphatase,s and phosphodiesterases activity. A deeper understanding of the functional effects of RyR2 phosphorylation is mandatory critical to developing new therapeutic tools to for improvinge the cardiac dysfunction and associated arrhythmias linked to HF.

RyR2 oxidation. Redox signalling also contributes to posttranslational modulation of RyR2.[181] During HF, cellular damage increases synthesis of reactive oxygen especies (ROS) and reactive nitrogen especies, synthesis leading to chronic oxidative stress with augmented cardiac demand.[182] Oxidative stress has been associated with elevated SR Ca^{2+} release[183] that

leads to abnormally elevated $[Ca^{2+}]_i$ in the myocardium during diastole.[184] Sulfhydryl groups of cysteine residues on RyR2 can be oxidized by ROS, producing sulfenic, sulfinic, and sulfonic acids via disulfide bond formation, *S*-nitrosylation, and *S*-glutathionylation.[185]

There is a general consensus that oxidation increases RyR2's activity,[186,187] while reduced oxidation leads to a less active channel;[188] however, several studies have indicated that the effects of oxidative agents towards RyR2 rely on experimental conditions,[189] pointing out that low concentrations of oxidizing agents activate RyR2, whereas prolonged exposure or elevated concentrations of oxidants leads to irreversible RyR2 inhibition.[190]

RyR2 oxidation has been shown to induce SR Ca^{2+} mishandling, arrhythmias, and contractile dysfunction in infarcted and failing hearts.[125,175,186,188,191–194] It has also been described in the pacing-induced HF canine model that carvedilol, a non-selective β-blocker with antioxidant properties, preserved ~~the~~ cardiac function by stabilizing the RyR2 structure and preventing its oxidation.[191] In ~~the~~ a canine model of chronic HF, ~~the~~ increased SR Ca^{2+} leak has been related to RyR2 oxidation.[195] Oxidation can also affect RyR2 intersubunit interaction, modifying RyR2 function and SR Ca^{2+} release.[196] A recent study pointed out that ~~the~~ redox-mediated RyR2 cross-linking has a significant impact on ~~the~~ channel activity and SR Ca^{2+} release, increasing the open probability of the channel and RyR2-mediated Ca^{2+} leak in ventricular myocytes isolated from a rabbit HF model.[196]

A growing body of evidence demonstrates a direct link between oxidative stress, RyR2 oxidation, and increased SR Ca^{2+} leak ~~during~~ in HF. In addition, both phosphorylation and redox modifications seem to have an additive effect on RyR2 function. In the non-ischemic canine HF model, RyR2 phosphorylation and thiol oxidation occur~~red~~ during HF: RyR2 phosphorylation by CaMKII takes place in the early stages of HF followed by RyR2 oxidation at later stages.[175] In failing heart tissue from patients with ischemic cardiomyopathy, ~~the~~ elevated SR Ca^{2+} leak was associated with RyR2 hyper-phosphorylation ~~on~~ at both PKA and CaMKII sites together with thiol oxidation.[197] Therefore, any therapeutic strategy for preventing HF-associated cardiac dysfunction and arrhythmogenesis will also require further insight~~s~~ into the molecular mechanisms ~~that participate in~~underlying RyR2 redox ~~regulation are essential for the development of specific and effective therapeutic strategies to prevent the cardiac dysfunction and~~ arrhythmogenesis ~~associated with HF~~.

3.4 Role of TRPs in HF

There is a general consensus that TRPs are expressed at very low levels in normal adult cardiac myocytes, but their expression and activity change significantly in pathological processes, such as HF (for review see [59]). Independent reports have established a clear link between the alteration of TRP isoforms activation and/or expression with cardiac hypertrophy and fibrosis as hallmarks of HF, as summarized in the Table.[56,198,199] For instance, a study using cardiac heart samples of patients at the with end-stage of HF demonstrated that TRP isoforms exhibit distinct expression profiles of expression in the left and right ventricles. Indeed, a significant increase of in the mRNA levels of TRPC1, C3, C4, and C6 is observed in the failing left and right ventricles;, and TRPV2 levels are similarly enhanced. In contrast, levels of TRPM2, M3, and M8 are reduced in the failing ventricles, as compared to non-failing control left and right ventricles.[200] Recently, Dragún and colleagues also showed significant increases in the expression of TRPC5, TRPM4 and M7 at the mRNA levels, but with downregulation of TRPC4 and TRPV2 in the myocardium samples of end-stage HF patients with who were end-stage HF candidates to for heart transplantation, as compared to those from healthy donors.[201] The authors also determined in rodents that of the TRP isoforms, only the expression of TRPC1 is strongly correlateds with the expression of the myocyte-enhancer factor 2c (MEF2c), a transcription factor implicated in cardiac hypertrophy and development.[202,203]

To unveil the critical role of TRP isoforms in cardiac hypertrophy and HF, several *in vivo* studies were performed in animal models, using knockout (KO) or transgenic mice, with or without specific procedures to stimulate elicit cardiac hypertrophy, includingas pressure overload induced by TAC, constriction of the pulmonary artery;, or in cardiac myocytes chronically treated with angiotensin-II, phenylephrine, endothelin-1, or aldosterone.[56,204–208].

However, the subunit composition of TRP channels in HF is still unknown; and TRPC, TRPV, or TRPM may participate in the formation of functional TRP channels underlying pathological cardiac remodeling. Future Additional studies are will be eagerly necessarycritical to determine the functional significance of these channels and its their transcriptional regulation in HF.

TRPCs. HF are associated withcan be induced by an o–verexpression of various TRPC isoformsStrategies aiming to induce HF promote the overexpression of different TRPC isoforms, which results ing in higher Ca^{2+} influx. Independent studies demonstrated that TRPC-induced Ca^{2+} influx activates such pro-hypertrophic pathways, as calcineurin/NFAT signaling, which initiates the expression of maladaptive hypertrophic genes, leading to HF.[56,69] For instance, TRPC1 KO mice showed werea prevention protected against of TAC-induced NFAT

activation and overexpression of ANP, BNP, and β-Myosin heavy chain (β-MHC), suggesting that TRPC1 plays a crucial role ~~of TRPC1~~ in cardiac hypertrophy induced by pressure overload.[209] Similarly, cardiac~~-~~-specific overexpression of dominant-negative (dn) TRPC3, C4, and C6 reduces SOCE, NFAT activation, and ~~heart~~ cardiac hypertrophy in the TAC mouse model.[69] Furthermore, TRPC1/C4 double KO mice ~~showed~~ exhibited similar ~~beneficial~~ protective effects ~~on~~ against pressure overload–induced hypertrophy and interstitial fibrosis.[205] Recently, the role of TRPC1 in cardiac myocyte~~s'~~ hypertrophy, associated with abnormal activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), was confirmed in TRPC1-KO human pluripotent stem cell lines generated by CRISPR/Cas9.[210] Moreover, it has been demonstrated that Pyr3, a specific inhibitor of TRPC3, reduces NFAT activation, ANP expression, and cardiac hypertrophy evoked by TAC.[211] Of note, gain-of-function transgenic models overexpressing TRPC3 acquire progressive cardiac hypertrophy,[212] or develop cardiomegaly and congestive HF in the case of TRPC6.[213] Interestingly, the overexpression of TRPC1, C3, and C6 observed in cardiac hypertrophy seems to promote their own expression, potentiating Ca^{2+} influx, NFAT activation, and the expression of hypertrophic genes (for review see [198]).

TRPMs. The role of TRPM isoforms in cardiac hypertrophy and HF has been also investigated. Morine et al. ~~TRPM4~~ used TAC and constriction of pulmonary artery animal models, which promote left and right ventricle overload, to demonstrate significant upregulation of TRPM3 and M7, although their mechanism of action was not addressed. ~~Generally,~~ TRPMs are generally ~~supposed~~ believed to play a protective role against HF. Indeed, TRPM4-KO mice show mild cardiac hypertrophy at 6 months~~,~~[214] and increased hyperplasia ~~in~~ as neonate~~sal~~, resulting in eccentric cardiac hypertrophy.[215] This concept has been also supported by data observed in cardiomyocyte-specific TRPM4-KO mice~~,~~ challenged ~~by~~ with chronic angiotensin II stimulation, in which cardiac hypertrophy parameters and the expression of pro-hypertrophic genes are increased compared to control.[216] ~~In this way~~Similarly, right ventricular pressure load evoked by monocrotaline treatment in rats also leads to a prominent downregulation of TRPM4 protein expression.[217] By contrast, a recent study demonstrated that TRPM4 inhibition by adeno-associated virus serotype 9 (AAV9)-mediated gene transfer improves cardiac contractility, suggesting that TRPM4 knockdown increases inotropic responses. However, this model has not yet been tested in an experimental model of HF.[218] Similar beneficial effects of TRPMs in HF ~~have been~~were observed in a study ~~in which~~with TRPM7 kinase-deficient mice, ~~that~~ which developed increased cardiac hypertrophy, fibrosis, and cardiac dysfunction after

chronic angiotensin II treatment, indicating that TRPM7 might play a protective role against angiotensin II effects.[219] On the other hand, angiotensin II stimulation of rat cardiac fibroblasts increases TRPM7 expression, increasing Ca^{2+} influx, NFAT activation, and α-SMA expression.[220] Therefore, TRPM4 and M7 could be promising targets to for improvinge cardiac responses in patients with HF, although their mechanism of action is still unclear.

TRPVs. TRPV1 and 2 are significantly upregulated in mice subjected to TAC.[221,222] TRPV3 is also overexpressed in angiotensin-II-induced cardiac hypertrophy, and is involved in calcineurin/NFATc3 signaling activation.[223] However, the molecular mechanism underlying the role of TRPVs in pathological cardiac hypertrophy remains unclear. Some studies determined reportedа reduced increase in heart weight and extracellular matrix remodeling in TRPV1-KO mice, compared to wild-type,[207] or in the absence of functional TRPV2,[222] under pressure-overload or physical exercise-induced cardiac hypertrophy, respectively. In contrast, others have suggested that *Trpv1* gene deletion promotes excessive inflammation and exacerbates cardiac hypertrophy after TAC, suggesting a protective role of TRPV1.[224] This benificial role of TRPV1 has been supported by studies using capsaicin, a specific TRPV1 agonist. In fact, dietary capsaicin attenuates the effects of pressure overload–induced cardiac hypertrophy and the increased cardiac fibrosis in wild type mice; however,, meanwhile, the benefits of capsaicin actions are not observed in TRPV1-KO mice.[225] Another study showed that capsaicin avoids circumvents high-salt diet-mediated cardiac hypertrophy by improving the mitochondrial complex I oxidative phosphorylation.[226] In contrast, oral delivery of TRPV1 antagonists reverses the loss-of- function in TAC-induced mice cardiac hypertrophy.[227] All In aggregate, these data pointed point to TRPs as new targets that with a significant role inly modulatinge the progression of HF.

4. NEW TARGETS FOCUSED OIN Ca^{2+} MISHANDLING LINKED TO HEART FAILURE

Increasing HF -research has been focused on the identification of new targets with a role in the management of Ca^{2+} handling and with a significance in for the progression of the disease. In this regard, postranslationals modificationsin of key regulators of Ca^{2+} handling such as O-GlcNAcylation have increased garnered the attention of a number of researchers. Furthermore, a research linea series of studies haves pointed to mediators of inflammationory and mineral metabolism mediators as potential new targets. with These mediators have a clear role in the

progression on HF, not only by virtue of ~~its~~ their immunological or mineral modulatory effects, but also by regulating intracellular Ca^{2+} dynamics and cardiac function.

4.1 O-GlcNAcylation.

In HF, cardiac cells undergo a metabolic shift in which they use~~ing~~ a predominantly glycolytic substrate rather than fatty acids (as compared to healthy cardiomyocytes). As such, ~~a more important fraction~~higher precentage of glucose goes through ~~the~~ accessory metabolic pathways such as the hexosamine biosynthesis pathway (HBP), leading to O-GlcNAcylation. O-GlcNAcylation is regulated by a rate-limiting enzyme, ~~the~~ glutamine-fructose-6-phosphate amidotransferase (GFAT). Similar~~ly~~ to phosphorylation, O-GlcNAcylation is a ~~fast~~rapid, ~~and~~ reversible addition of a UDP-O-GlcNAc group to Ser and Thr residues. ~~Contrarily~~ Unlike ~~to~~ phosphorylation, which involves a plethora of kinases, O-GlcNAcylation is regulated ~~by~~ by only two enzymes: ~~two enzymes only, not a plethora of kinases, which are the~~ O-GlcNac transferase (OGT), ~~that~~ which adds the O-GlcNAc group, and ~~the~~ O-GNAcase (OGA), which ~~that~~ removes it. Recently, the post-translational modification O-GlcNAcylation has emerged as a key player in HF, including ~~in~~ with protein targets controlling Ca^{2+} handling. Studies have shown that cardiac O-GlcNAcylated protein levels increase~~s~~ in common etiologies of HF such as diabetes, hypertension, aortic stenosis, and myocardial infarction in both human and animal models.[228–232]

Most of our knowledge ~~on~~ about the adverse effects of O-GlcNAcylation ~~adverse effects~~ on ~~heart~~ cardiac and cardiomyocyte~~s~~ function has been ~~deciphered~~ garnered ~~in~~ from HF models with diabetic aetiology or in cells treated with high glucose and glucosamine, a precursor of the HBP.[228,233] ~~In the diabetic rodent model,~~ Eexpression of key markers of O-GlcNAcylation, such as OGT and GFAT, is increased over time in the diabetic rodent model.[234] Moreover, Ca^{2+} handling is altered, with a prolonged Ca^{2+} transient decay time associated with SERCA2 down-regulation (mRNA and protein) and a decrease of PLB phosphorylation. Interestingly, adenoviral overexpression of ~~the~~ OGA~~,~~ prevents or significantly reduces Ca^{2+} mishandling and improves ~~the~~ contractile cardiac function.[233] In an HF mouse model induced by TAC, ~~while~~ OGT deletion exacerbate~~d~~s cardiac dysfunction and fibrosis,[235] although SERCA2 expression levels were unchanged and both PLB and troponin phosphorylation decreased.[235] Contractile dysfunction in ~~–~~an experimental model with high O-GlcNAcylation levels has also been attributed to ~~a~~ decreased Ca^{2+} sensitivity in ~~Ca^{2+}~~ myofilaments. Indeed, in a type 1 diabetic rat model ~~with, similarityly to humans~~, both OGT and OGA undergo delocalization and changes in activity. Interestingly, removal of myofilament O-GlcNAcylation~~,~~ ~~—~~using a bacterial analog

of OGA, restores Ca^{2+} sensitivity in the streptozotocin-induced diabetic rat.[236] Furthermore, O-GlcNAcylation of cardiomyocytes seems to be involved in ventricular arrhythmogenic mechanisms observed in the progression of HF. The link between O-GlcNAcylation and arrhythmia susceptibility was established in a study by Erickson et al., in which hyperglycemic conditions activated CaMKII, a key protein involved in HF and cardiac arrhythmia. Indeed, the authors found that in cardiomyocytes treated with high glucose, CaMKII undergoes O-GlcNacylation at Ser279. The direct activation of CaMKII results in increased diastolic Ca^{2+} release and exacerbation of arrhythmic events in diabetic rats under β-adrenergic stress.[237] The O-GlcNAc activation of CaMKII activates NOX2 and cytosolic production of reactive oxygen species, which could trigger ventricular cardiac arrhythmia.[238] Finally, in high glucose conditions or in diabetic rat models, O-GlcNAcylation leads to a redistribution of Nav1.5 to the cytosol and a decrease in its expression at the surface membrane, reducing the Na^+ current and increasing late Na^+ current. This alteration of Na^+ channel function is associated with a prolongation of the AP and susceptibility to cardiac arrhythmias.[239] One of the weakness of most studies lies with the diabetic etiology, which could alter EC coupling on its own. Indeed, it is commonly acknowledged that obesity, insulin-resistance, and inflammation state, all of which are associated with diabetes, contribute to the alteration of EC coupling.[240–242] With our current knowledge, it has not been conclusively established whether O-GlcNAcylation plays a causal role in pathology or whether it is a consequence of pathological stress. In this vein, a recent study sought to solve this issue by generating transgenic mouse models with myocardial overexpression of OGT to control O-GlcNAcylation independently of any pathological stress.[243] Interestingly, solely the increase of O-GlcNAcylation lead to severe dilated cardiomyopathy with reduced left ventricular ejection fraction, and increased left ventricular diameter at 6 weeks, ventricular arrhythmias, and premature death through impairment of mitochondrial complex I activity. However, aside from a low diastolic Ca^{2+}, the other components of Ca^{2+} signalling, such as Ca^{2+} transients and SR load, were not affected by the OGT overexpression. In pathological conditions such as ischemic HF, the decrease of troponin T phosphorylation at Ser208 is associated with an increased troponin T O-GlcNAcylation of troponin T at Ser190, showing an interplay between phosphorylation and O-GlcNAcylation of sarcomeric proteins in HF.[244,245] Although increasing evidence highlights a key participation role for O-GlcNAcylation in the pathology of HF and its progression to ventricular cardiac

arrhythmia, our understanding of the underlying mechanisms and ~~its~~ regulation in HF independent~~ly~~ of diabetes is rather limited and, thus, further studies are ~~still~~ needed.

4.2 The innate immune system, inflammation and Ca^{2+} handling

The innate immune system is the first mechanism for host defense against exogenous and endogenous ~~dangers~~threats. It has the ability to develop an adaptive response, but also to perform specific mechanisms that lead to inflammatory responses in order to fight and resolve the ~~danger~~threat. Classically, the innate immune system was thought to recognise pathogens but, in ~~the last~~recent decades, ~~evidence~~ studies ha~~ve~~s revealed that ~~the~~ innate immune receptors are also able to recognise endogenous danger signals. These receptors,~~,~~ known as damage-associated molecular pattern~~s~~ (DAMPs), ~~that~~ activate the innate immune response by recruiting immune cells and initiating the production of pro-inflammatory cytokines. The machinery responsible for detecting these DAMPs and triggering the immune response are the pattern recognition receptors (PRRs), which include ~~ among others~~ toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), among others. Within the NLRs, the NOD~~s~~ and NLR~~s~~ with a pyrin domain (NLRPs) subfamilies are of great importance in HF.

Several studies have documented the important role of both TLRs and NLRs in patients with coronavirus disease 2019 (COVID-19) ~~patients~~.[246–248] It has been shown that a disruption in immune system regulation increases the risk of adverse outcomes in patients with COVID-19-related cardiovascular disease.[249,250] Thus, given the key role of innate immunity in cardiovascular diseases and its implication in COVID-19, a deeper understanding of the interplay between both the cardiovascular and innate immune system during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection~~,~~ might provide novel therapeutic opportunities for the treatment of this pathology and particularly for the associated cardiovascular complications.

~~Largely studied,~~ TLRs are widely studied and represent~~,~~ the ~~most well~~best-known PRRs;~~,~~ ~~are~~ they are primarily expressed ~~mainly~~ in immune cells, but also in other cell types such as cardiomyocytes. The expression of NLRPs and NODs is ubiquitous in adult tissues;~~,~~ for example, NOD1 is expressed both in innate immune cells ~~as well as~~and in cardiomyocytes and fibroblasts. In healthy tissue, PRRs are involved in the maintenance of tissue homeostasis. ~~In~~ Over the last decade, the innate immune system has emerged as a major player in the ~~setting and development~~ progression of cardiovascular diseases.[251–258] Interestingly, HF is frequently developed after myocardial infarction or chronic metabolic stress, leading to ~~a~~ progressive

damage of cardiac tissue and provoking the release of proinflammatory cytokines and DAMPs, which activate the innate immune response through PRRs.[259–261] A~~The a~~ctivation of PRRs induces the release of several proinflamatory mediators, such as IL-1β~~-~~, that can exert harmful cardiac effects. In this ~~line~~vein, a recent study ~~of~~ by Liu et al. ~~in~~ used a high-fat diet diabetes type 2 mouse model to demonstrate~~d~~ a link between increased IL-1β expression in the heart, increased mitochondrial oxidation, and augmented spontaneous Ca^{2+} leak from the SR, leading to early after depolarizations and arrhythmias.[262] Among cytokines, IL-1β is upstream in the inflammation pathway and directly implicates innate immune system in ~~the deletereus~~deleterious cardiac remodelling. Indeed, Monnerat et al. demonstrated that, in a mouse model of diabetes type 1, ~~the~~ depletion of TLR2 and NLRP3 in heart macrophages ~~is able to~~ reduced IL-1β expression and prevented deleterious cardiac electrical remodelling.[263]

It has been reported that HF patients have elevated circulatory levels of TNF-α, IL1-β, and other inflammatory cytokines, which are directly related to the severity of HF progression.[264–267] R~~Some r~~ecent clinical trials, such as the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) and the Colchicine Cardiovascular Outcomes Trial (COLCOT), support ~~that~~ the notion that specific anti-inflammatory treatments improve the condition and prevent mortality in patients with cardiovascular diseases.[268,269]

Indeed, novel studies ~~are~~ have recently elucidate~~ding~~ the role of PRRs in cardiac EC coupling and HF progression.[92,270–274] Classically, TLR~~s~~ activation has been largely related to deleterious alterations in cardiac function after myocardial infarction~~;~~,[275] ~~and~~ elevated TLRs expression was found in patients ~~who suffered from~~following myocardial infarction.[276,277] Studies have revealed that TLR2 or TLR4 deficiency attenuates myocardial inflammation, reducing ~~the~~ infarct size~~,~~ and preventing ventricular dysfunction after ischemia/reperfusion injury in mice.[278–283] Moreover, the deleterious cardiac ~~deleterious remodell~~remodeling observed in these models was associated with Ca^{2+} handling impairment. For instance, several studies have reported that, upon lypopolysaccharide (LPS) stimulation in rat ventricular cardiomyocytes, TLR4 activation triggers action potential prolongation and increases Ca^{2+} efflux through NCX channels, promoting pro-arrhythmogenic events.[284–286] Likewise, TLR4 can ~~also~~ be activated by the inflammatory cytokine high-mobility group ~~-~~box 1 (HMGB1) subsequently leading to ROS overproduction and oxidative stress.[287,288] In this sense, the blockage of TLR4/ROS signaling appears to prevent the enhanced SR Ca^{2+} ~~-~~leak caused by HMGB1, restoring the depleted SR Ca^{2+} ~~-~~load, amplitude of ~~systolic~~ Ca^{2+} ~~-~~transients, and contractility in adult rat ventricular myocytes.[289] However, the restored cardiac function after

TLR4 inhibition seems to be partial, indicating that, not only TLRs, but also other mechanisms are implicated in the cardiac Ca^{2+} remodeling induced by HMBG1.

The family of NLRPs is associated with inflammasomes, which are macroprotein complexes that activate caspase-1, leading to production of pro-inflammatory cytokiness production such as IL-1β, IL-18, and HMGB1.[290,291] The activation of the NLRP3 inflammasome is caused by cellular damage indicators including Ca^{2+} mobilization and mitochondrial dysfunction.[292] Furthermore, Ca^{2+} -signaling has been suggested as a key regulator of NLRP3 inflammasome.[293] In this regard, Ca^{2+}-sensing receptor (CaSR) has been reported to activate phospholipase C (PLC), which generates inositol triphospate IP_3. IP_3, in turn, that links to the IP_3R channel, in turn, inducing SR Ca^{2+} leak and activating NLRP3, which contributesing to cardiac dysfucntion.[294,295] Interestingly, NLRP3 inflammasome activation has been also related to RyR2 over-expression in mouse NLRP3-overexpressed cardiomyocytes, increasing SR-Ca^{2+} leak, altering Ca^{2+} handling and triggering pro-arrhythmogenic events.[296] In contrast, the genetic deletion of *Nlrp3* has been shown to reduce the incidence of atrial fibrillation (AF).[274,297] Moreover, Byrne et al., have demonstrated that empagliflozin, a SGLT2 inhibitor prevents cardiac inflammation by attenuating the activation of the NLRP3 inflammasome in a Ca^{2+}-dependent manner, exerting beneficial effects in a rodent model of HFrEF.[298]

Finally, the NODs constitute another subfamily of NLRs that starts to rise asgaining notoriety as key playerss in aberrant Ca^{2+} handling in associated with cardiovascular diseases. Specifically, NOD1 has been associated with several diseases that have with a detrimental cardiovascular outcome.[92,299–303] In this regard, NOD1 activation aggravates cardiac damage after ischemia/reperfusion injury, increasing infarct size, cardiomyocyte apoptosis, and inflammation in murine HF models.[304] Specifically, upon NOD1 activation with the specific agonist iE-DAP, diminished I_{CaL} density, depressed Ca^{2+} transients, and slower time decay of Ca^{2+} transient decay s were found in cardiomyocytes, all of which promote ing depressed cardiac outcome in mice.[271] In addition, *Nod1* genetic deletion prevented cardiac dysfunction in a murine HF model with permanent coronary artery ligation, improving systolic Ca^{2+}-release, restoring SR Ca^{2+} load, and consequently reducing the occurrence of pro-arrhythmogenic events, all these all effects that contribute to improved the cardiac function in failing mice.[92] Importantly, these Ca^{2+} alterations were also reversed and pro-arrhythmogenic events were diminished when HF mice were treated with a pharmacological inhibitor of NOD1.[92] Moreover, *Nod1* deletion also prevented Ca^{2+} mishandling, maintaining the amplitude of the Ca^{2+} transients amplitude, SR Ca^{2+} load, and reducing the incidence of

spontaneous Ca^{2+} release during diastole under β-adrenergic stimulation in failing cardiac murine cardiomyocytes.[273] Remarkably, supporting the observed results in experimental models, high expression of NOD1 was also found in cardiac tissue from severe ischemic HF patients.[92]

In light of these recent studies, ~~the~~ PRRs of the innate immune system are emerginge as crucial factors in the regulation of intracellular Ca^{2+} handling in cardiac EC coupling (Panel I, Figure 2). The innate immune system and cellular Ca^{2+} dynamics create a vicious cycle between Ca^{2+} sensing-Ca^{2+} mishandling and pro-inflammatory signaling that leads to cardiac dysfunction and finally to HF development. ~~Herein~~Therefore, innate immune receptors ~~stand~~ offeras ~~a new~~ a promising avenue ~~hub~~ for new therapeutic targets ~~for~~ to treat Ca^{2+} mishandling and cardiac function impairment in HF.

4.3 Mineral bone metabolism factors as a new axis involved in HF-Ca^{2+} mishandling

Classically, profound disturbances in mineral and bone disorders have been almost exclusively linked to chronic kidney disease (CKD). However, over the last decade, clinical and experimental evidence ~~from the last decade reveals~~has revealed that alterations in mineral bone homoeostasis ~~have~~ also have a strong impact on the heart. This could be due to ~~the~~ direct and indirect heart-kidney bidirectional interactions, encompassing a spectrum of disorders with a complex etiology~~ntity~~ classified as cardiorenal syndrome (CRS). CRS is defined as an acute or chronic dysnfunction in the heart or kidneys ~~which~~ that may induce acute or chronic dysfunction in the other organ.[305] Between the candidate factors proposed to play a relevant role in this cardiorenal connection are those involved in ~~the~~ mineral bone metabolism, such as the axis fibroblast growth factor (FGF)-23 and Klotho.[306] FGF-23 is considered an endocrine phosphaturic hormone; it ~~which~~ is synthesized in osteocytes and osteoblasts ~~as~~ with declining renal function ~~declines~~ to increase renal phosphorus excretion and reduce systemic phosphate accumulation.[307,308] It is well known that FGF-23 systemic levels increase as renal function declines;~~,~~ ~~and~~ high levels of FGF-23 are also associated with increased risk of cardiovascular disease, adverse cardiovascular outcomes, ~~and~~ death in patients with or without CKD;~~,~~[309,310] ~~being~~ indeed, FGF-23 ~~nowadays~~ is now considered ~~as~~ a relevant cardiorenal mediator. In this ~~sense~~vein, several authors have shown a clinical relationship between high levels of FGF-23 and HF.[311–314] Despite these relevant clinical data, little is known ~~regarding~~ about the involvement of FGF-23 in the regulation of ~~the~~ cellular cardiac function. Several authors have

shown that *in vitro* exposure of ventricular adult cardiomyocytes to FGF-23 induces important changes in Ca^{2+} handling.[315,316] ~~In this sense~~In support of this finding, FGF-23 significantly increases $[Ca^{2+}]_i$ in primary ventricular cardiomyocytes,[315] ~~,~~ which could trigger pro-hypertrophic pathways in the long-term, thus explaining its specific clinical association with ~~the presence of~~ left ventricular hypertrophy in patients with CKD.[317] Moreover, ~~the~~ increase in $[Ca^{2+}]_i$ after FGF-23 exposure is explained by the specific FGF-23 actions on RyR2s in adult ventricular cardiomyocytes.[316] Acute *in vitro* exposure to FGF-23 induces a significant increase in spontaneous diastolic Ca^{2+} leak from SR in the form of Ca^{2+} sparks and waves, along with ~~and a~~ decreased ~~in~~ systolic Ca^{2+} transients and SR-Ca^{2+} load, thus compromising cardiomyocyte contraction. Moreover, acute FGF-23 exposure triggers *in vitro* pro-arrhythmogenic activity such as spontaneus~~automatic~~ ~~systolic~~ Ca^{2+} transients and extra-contractions in isolated cardiomyocytes and rhythm alterations recorded *in vivo* by electrocardiogram as premature ventricular contractions in mice.[316] Few studies ~~are~~ have ~~focusing~~ examined ~~on~~ the underlying functional mechanisms downstream FGF-23 in adult ventricular cardiomyocytes. Among the mechanisms underlying FGF-23 effects in adult ventricular cardiomyocytes are the calmodulin~~e~~ ~~quinase~~ kinase type II (CaMKII)- and phosphodiesterase 4B (PDE4B)-dependent pathways, both of which are involved in HF.[316,318] FGF-23 promotes phosphorylation of RyR2s at the CaMKII site Ser^{2814} ~~, supporting~~ exerting its ~~actions~~ effects on Ca^{2+} leak from SR through RyR2s via the CaMKII-dependent pathway in isolated ventricular adult cardiomyocytes.[316] More recently, other authors have ~~also~~ shown that FGF-23 is able to increase the frequency of Ca^{2+} waves, ~~as~~ a marker of cellular arrhythmogenicity, in adult cardiomyocytes ~~by~~ through acute beta-adrenergic stimulation secondary to a decrease in PDE4B levels.[318] The involvement of both pathways has been recently corroborated in 5/6 nephrectomized mice,[318,319] an established experimental model of uremic cardiomyopathy that ~~curses with~~exhibits systemic, maintained elevation of FGF-23 levels and profound intra-cardiomyocyte Ca^{2+} mishandling.[319,320] T~~All t~~hese experimental data support the close relationship between high FGF-23 levels and the predisposition to arrhythmias, ~~proposing~~ highlighting FGF-23 as a new potential therapeutic target. Therefore, blocking the deleterious actions of FGF-23 on the heart might reduce the adverse cardiac outcomes observed in these pathologies, especially ~~in~~ those ~~that also curse~~ with mineral bone disturbance~~s~~ and renal failure co-morbidities.

On the other hand, Klotho is one of the most important factors involved in the control of mineral bone metabolism.[321] Klotho is mainly synthesized in the kidneys, where it binds to FGF

receptors, enhancing their ~~its~~ affinity for ~~the~~ circulating FGF-23 and promoting renal phosphate excretion. It is well established that renal failure ~~curses~~ coincides with a progressive reduction in Klotho expression, compromising phosphate excretion. In addition to the main physiological action of membrane-bound Klotho ~~at~~ in ~~the~~ renal tissue, Klotho is also shedded by secretases into circulation as a soluble form ~~which~~ that can exert other off-taget actions; for example, it reportedly has cardioprotective effects in ~~as on~~ the heart ~~where cardioprotection actions have been described~~.[306] The first evidence ~~showing~~ identifying Klotho as a regulator of Ca^{2+} handling ~~was described~~focused on TRPC6. Klotho-deficient mice showed exaggerated cardiac hypertrophy and deleterious remodelling in response to stress mediated by ~~the~~ specific cardiac downregulation of ~~the~~ TRPC6, reducing Ca^{2+} entry through ~~this~~ these Ca^{2+}-permeable cation channels.[322] Moreover, soluble Klotho also blocked TRPC6 current via ~~-~~ the phosphoinositide-3-kinase-dependent (PI3K) pathway in cardiomyocytes.[322] More recently, several authors have also shown the cardioprotectiveon ~~actions~~ effects of Klotho through the regulation of other proteins that participate in Ca^{2+} handling. In addition, *in vitro* experimental approaches have ~~described~~ demonstrated that soluble Klotho inhibits ~~the~~ deleterious FGF-23 actions on RyR2s, preventing ~~the~~ pro-arrhythmogenic Ca^{2+} leak, impeding ~~the~~ CaMKII-dependent phosphorylation at Ser^{2814} ~~a~~, and preventing FGF-23-induced PDE4B decreased or PDE3A and 3B increased expression in the absence of FGF-23 in adult ventricular cardiomyocytes.[316,318] Interestingly, enhancing Klotho availability~~,~~ either ~~by~~ through supplementation with exogenous recombinant Klotho ~~supplementation~~ or by using transgenic mice with Klotho overexpression~~,~~ improves cardiac function via regulation of Ca^{2+} handling in HF conditions linked to uraemic cardiomyopathy.[319] Similarly, adult cardiomyocytes from hypomorphic Klotho mice, which present ~~a highly~~ extremely strong elevation of systemic FGF-23 levels, showed a decrease in intracellular Ca^{2+} transients and cellular shortening together with an increase in pro-arrhythmic Ca^{2+} events.[319] These experimental results could explain why elevated levels of circulating Klotho are associated with a lower risk of developing cardiovascular disease after adjusting for traditional cardiovascular risk factors, as observed in elderly individuals.[323]

Taken together, ~~All this relevant and recent~~this evidence supports the role of the FGF-23 and Klotho axis as a novel bone-heart-kidney regulator of cardiac Ca^{2+} handling (Panel II, Figure 2). However, further experimental studies are ~~still~~ needed to fully ~~decode~~ elucidate the underlying mechanisms by which these mineral bone factors impair Ca^{2+} handling and cardiac function, going beyond the confines of nephrology and cardiology. ~~In this sense, it has also~~

been shown that FGF-23 levels can be slightly increased under other circumstances such as variations in phosphate intake or diet, and Klotho levels have been found to be associated with aging.

5. REMARKS AND CONCLUSIONS

Besides the large enormous body of literature available information regardingconcerning the role of Ca^{2+} handling in the pathogenesis of different forms and stages of HF, many questions remain openedunanswered. Basic scientistss and clinician's researchers are still looking in search of for new therapeutic tools to improve the poor prognosis of patients with HFcardiac failing patients. In this scenarioscientific milieu, a deeper knowledge understanding of post-translational changes in key proteins of involved in Ca^{2+} regulation, including phosphorylation, oxidation, or O-GlcNAcylation, is today currently the main primary focus of many researchs studies that try to understandseeking to unravel the intrinsic mechanisms involved in this complex disease. On the other hand, new mediators related to mineral bone metabolism regulation, such as FGF-23 or Klotho, have emerged as new modulators of the EC coupling, with an interesting role in the Ca^{2+} mishandling linked to HF. Finally, mediators of the innate immune system, which with have a clear role in the inflammatory response, have increased theirgained interest in the field of cardiovascular diseases, including HF. In this regard, NLRs such as NLRP3 or NOD1 are emerging as promising new targets for cardiac complications, enabling thein the development of more specific HF -therapies.

Legends to fFigures legends

Figure 1. Excitation-contraction coupling in the heart. ***Panel I) Ca^{2+} handling in healthy hearts.*** After membrane ventricular cardiomyocyte depolarization, **1)** Ca^{2+} enters the cardiomyocyte through LTCCs, **2)** the small Ca^{2+} influx triggers RyR2 opening, releasing enough sufficient Ca^{2+} from the SR to the cytoplasm, and to **3)** triggering cell contraction. **4)** During relaxation, Ca^{2+} is removed from the cytoplasm mainly primarily by the SERCA2a pump, which introduces resequesters Ca^{2+} back into the SR lumen; also, Ca^{2+} is also extruded from the cell by the NCX, which that introduces Na^{+} at the same time; while finally, a small amount of Ca^{2+} is taken up by the mitochondria. **5)** SERCA2a activity is regulated by PLN, which, in its unphosphorylated state, is bound to SERCA2a, inhibiting its activity. When PLN

is phosphorylated by PKA or CaMKII, it detaches from SERCA2a, augmenting its activity. **6)** Additionally, β-adrenergic stimulation activates kinases such as PKA and CaMKII that phosphorylate different key EC coupling proteins including RyR2, LTCC, and PLN, modifying their activity. Finally, **7)** other types of Ca^{2+} channels participate in EC coupling such as the TRPCs, which, in combination with Orai1 and STIM1, introduce Ca^{2+} to the cell when STIM1 senses that SR Ca^{2+} levels are low. ***Panel II) Ca^{2+} handling in heart failure.*** In failing ventricular cells, **1)** T-tubule structural alterations disturb the dyadic space and ~~dysregulate~~ disrupt the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism; **2)** β-adrenergic receptors initiate G-protein signaling that activates adenylyl cyclase (AC), transforming ATP into cAMP, which activates PKA and CaMKII. These, in turn, phosphorylate~~ing~~ key Ca^{2+} channels such as LTCCs and RyR2 (at S^{2008}, S^{2814}, and S^{2030}). RyR2 phosphorylation increases ~~its~~ the channel's open probability, leading to enhanced SR Ca^{2+} release and increased cytosolic intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). **3)** Increased $[Ca^{2+}]_i$ promotes mitochondrial Ca^{2+} dysregulation, ~~that~~ which leads to mitochondrial-ROS overproduction and oxidative stress conditions. This oxidative environment can also favor post-transcriptional modifications of RyR2, altering its conformation;~~,~~ all of ~~them~~ these contribute~~ing~~ to Ca^{2+} mishandling. **4)** HF is characterized by low expression of SERCA2a, leading to reduced SR Ca^{2+} load, and~~,~~ **5)** ~~it~~ this is accentuated by reduction in the phosphorylation levels of PLN, which, in its unphosphorylated state, inhibits SERCA2a and reduces the amplitude of Ca^{2+} transients during systole. **6)** As the cardiomyocyte tries to restore ~~the~~ physiological Ca^{2+} homeostasis, the NCX augments its expression and extrudes more Ca^{2+} in exchange for Na^+-, favoring a depolarizing Na^+ current, which can lead to pro-arrhythmogenic events. **7)** Moreover, TRPC channels are overexpressed in HF, increasing the Ca^{2+} current that enters the cardiomyocyte.

Figure 2. New targets in Ca^{2+} mishandling linked to heart failure. ***Panel I) Innate immune system factors in HF-Ca^{2+} mishandling.*** **1)** TLR4 activation triggers AP prolongation and promotes enhanced SR Ca^{2+} leak and~~,~~ **2)** increases Ca^{2+} efflux through NCX channels leading to pro-arrhythmogenic events.~~,~~ **3)** TLR4 can also ~~produced~~ increase~~d~~ ROS production and, ~~subsequently~~consequently, increased oxidative stress. **4)** NLRP3 is associated with inflammasomes that lead~~s~~ to production of pro-inflammatory cytokines ~~production~~ such as IL-1β, IL-18 and HMGB1. TLR4 can also be activated by HMGB1, subsequently leading to ROS overproduction and oxidative stress.~~,~~ **5)** NLRP3 inflammasome~~s~~ cause~~s~~ mitochondrial dysfunction.~~,~~ **6)** NLRP3 leads to SR Ca^{2+} leak and RyR2 overexpression, altering Ca^{2+} handling and leading to pro-arrhythmogenic events.~~,~~ **7)** NOD1 activation diminish~~es~~ I_{CaL} density and

reduced systolic Ca transients, **8)** reduced SR Ca^{2+} load, and increased pro-arrhythmogenic events. ***Panel II) Mineral bone metabolism factors in HF-Ca^{2+} mishandling.*** **1)** FGF23 increases the intracellular Ca^{2+} concentration, which could can trigger pro-hypertrophic pathways. **2)** The FGF23 signaling pathway is CaMKII-dependent, promoting phosphorylated CaMKII, PLB in at Thr17, and RyR2 in at Ser2814, **3)** significantly increasing diastolic spontaneous Ca^{2+} leak from the SR, and decreasing Ca^{2+} transients and SR Ca^{2+} load, **4)** contributing to increased pro-arrhythmogenic events and leading to reduced contractility. **5)** Soluble Kklotho (sKlotho) blocks TRPC6 channels and **6)** inhibits the deleterious effects of FGF23's actions on RyR2, preventing the pro-arrhythmogenic Ca^{2+} release and CaMKII phosphorylation.

	TRP Channel Expression	**Main mechanism**
Human end-stage Heart Failure		
	• Upregulation of TRPC1, C3, C4, C6 and TRPV2. Downregulation TRPM2, M3, and M8.[200] • Upregulation of TRPC1, C5, TRPM4, and M7. Downregulation of TRPC4, and TRPV2.[201]	• Samples from left and right human ventricles. SourceUnveiled mechanism.[200] • Samples from left human ventricles. TRPC1 overexpression correlates with MEF2c.[201]
Study model of Heart Failure		
	TRPC channels Left and right ventricle overload animal model presented significant upregulation of TRPC1, C4, C3, and C6 (reviewed [56,69,198,199,204]).	• Exacerbated Ca2+ influx, activation of calcineurin/NFAT signaling pathway, expression of hypertrophic genes (reviewed [56,69,198,199,204]). • KO of TRPC1, and double KO of TRPC1/C4 attenuate TAC-induced hypertrophy and fibrosis.[205,210] • Inhibition of TRPC3, C4, and C6 reduces hypertrophy.[69,211] • Overexpression of TRPC3 and TRPC6 promotes hypertrophy and HF, respectively.[212,213]
	TRPM channels • Left and right ventricle overload promote the overexpression of TRPM3 and M7.[200] • Monocrotaline-induced right ventricle overload induced TRPM4 downregulation.[217]	• TRPM4 KO develop cardiac hypertrophy.[214–217] • TRPM7 deletion stimulates HF after angiotensin-II treatment.[219]
	TRPV channels • Left and right ventricle overload promote the upregulation of TRPV2 and V4.[200,221,222]	• TRPV1 KO exacerbates TAC's deleterious effects.[224] • TRPV1 KO[207] and V2 inhibition[222] prevent cardiac hypertrophy.

	• TRPV3 overexpression in angiotensin II induced hypertrophy.[223]	• Capsaicin activation of TRPV1 attenuates TAC effects.[225] • TRPV3 stimulates calcinuerin/NFATC3 signaling.[223]

Table 1. Summary information related to the expression of TRP channels and their related effects in patients with end stage heart failure (HF) and in animal models of left and right induced HF. KO, knock out; MEF2c, myocyte enhancer factor 2c; TAC, transverse aortic constriction.

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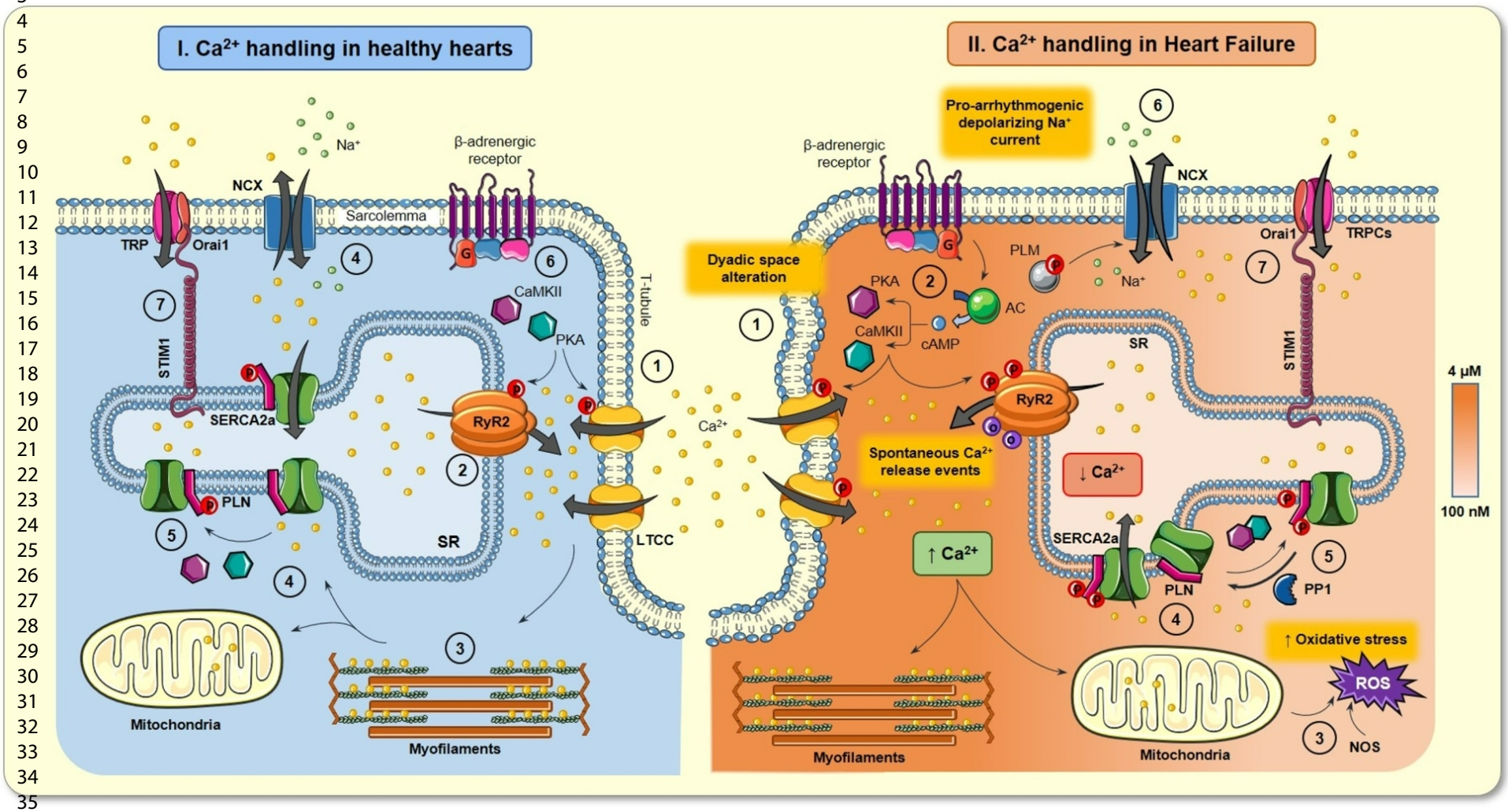
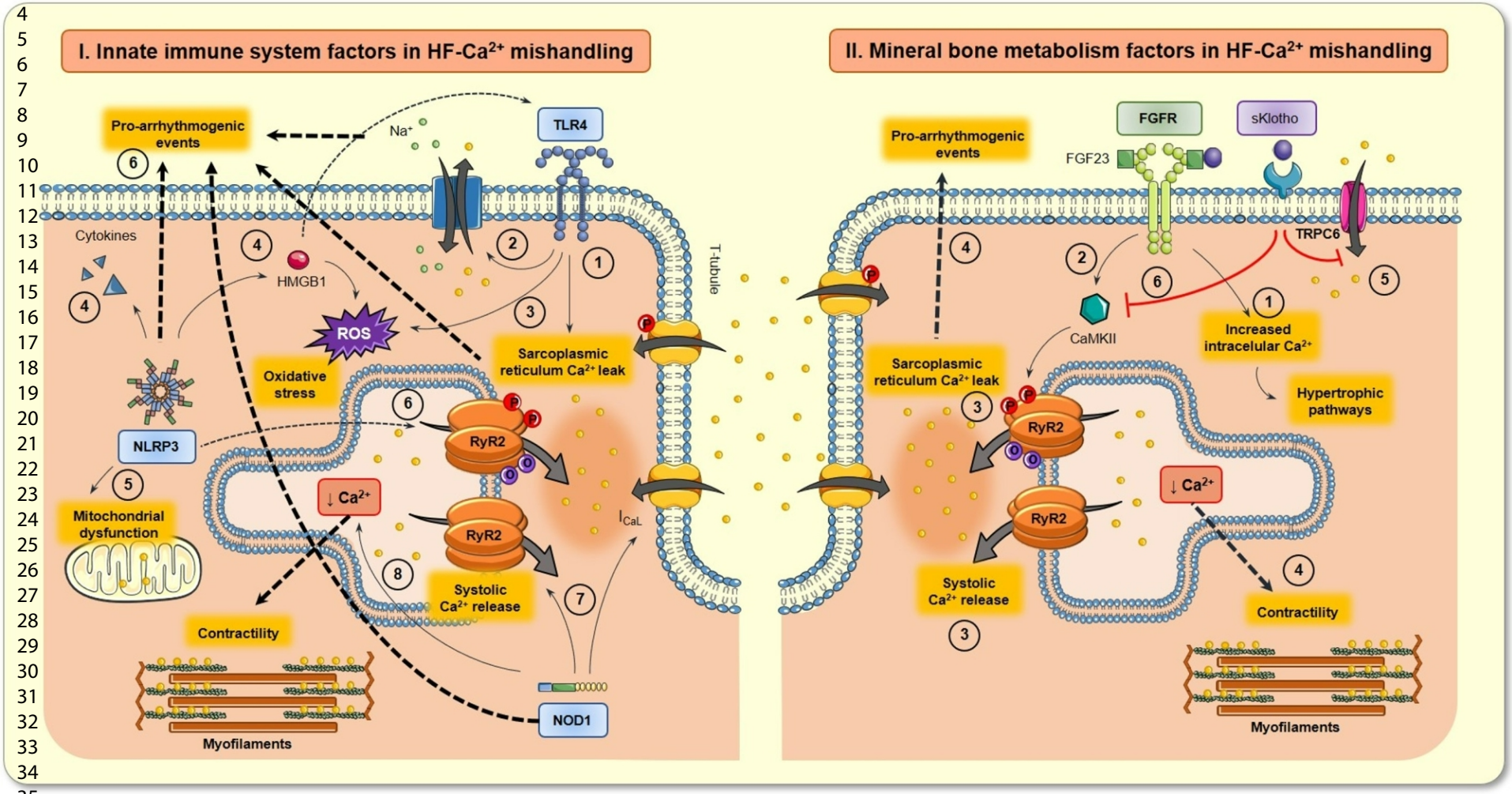

I. Ca2+ handling in healthy hearts
II. Ca2+ handling in Heart Failure
Na+
NCX
Sarcolemma
β-adrenergic receptor
TRP
Orai1
STIM1
SERCA2a
RyR2
PLN
PKA
CaMKII
T-tubule
LTCC
SR
Ca2+
Mitochondria
Myofilaments
Pro-arrhythmogenic depolarizing Na+ current
Dyadic space alteration
PLM
AC
cAMP
TRPCs
Spontaneous Ca2+ release events
↓ Ca2+
↑ Ca2+
PP1
↑ Oxidative stress
ROS
NOS
4 µM
100 nM


I. Innate immune system factors in HF-Ca2+ mishandling
Pro-arrhythmogenic events
6
Na+
TLR4
Cytokines
4
HMGB1
4
2
1
3
ROS
Oxidative stress
Sarcoplasmic reticulum Ca2+ leak
NLRP3
6
RyR2
P
O
5
Mitochondrial dysfunction
↓ Ca2+
RyR2
ICaL
T-tubule
8
Systolic Ca2+ release
7
Contractility
Myofilaments
NOD1
II. Mineral bone metabolism factors in HF-Ca2+ mishandling
Pro-arrhythmogenic events
FGFR
sKlotho
FGF23
TRPC6
4
2
6
5
1
CaMKII
Increased intracelular Ca2+
Sarcoplasmic reticulum Ca2+ leak
Hypertrophic pathways
3
RyR2
P
O
↓ Ca2+
RyR2
Systolic Ca2+ release
4
Contractility
3
Myofilaments