*International Journal of*
*Drug Discovery and Pharmacology（IJDDP）*

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*Review*

# Transverse Tubules in Cardiac Health and Disease: Therapeutic Approaches to T-Tubule Remodelling and Heart Failure Management

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Received: 24 July 2024; Revised: 10 September 2024; Accepted: 10 September 2024; Published: 3 December 2024

**Abstract:** Transverse tubules are specialised invaginations of the cardiomyocyte sarcolemma. They play a crucial role in cardiac cells by facilitating the coordination of calcium signalling and contraction. They achieve this by housing many ion channels and establishing connections with important structural elements within the cell. The morphology of t-tubules exhibits significant variation across different species and within distinct regions of the heart, allowing for the customisation of their functionality to suit the individual physiological requirements of the organism. Smaller animals with higher heart rates possess more intricate and densely concentrated t-tubules in comparison to larger mammals. This indicates the need for fast calcium signalling. In heart failure, there are harmful alterations in the t-tubules. These changes include a reduction in their density and a rearrangement from their usual alignment. As a result, the heart's ability to control calcium levels is compromised. As a result, there is a decrease in the strength of heart contractions and a lack of efficient relaxation, which both contribute to the symptoms and progression of the condition. This paper examines the present medications and interventions that specifically address t-tubule remodelling, which have demonstrated efficacy in restoring certain elements of t-tubule function. Additionally, it emphasises the capacity of physical activity to enhance t-tubule density and general cardiovascular well-being. Recent advances in imaging technology provide intriguing opportunities to investigate t-tubules in live cells. These observations have the potential to result in substantial enhancements in the therapy and control of heart failure.

**Keywords:** transverse tubules; calcium signalling; heart failure; T-tubule remodelling; therapeutic interventions; superresolution microscopy

## 1. Introduction

The cardiomyocyte sarcolemma is permeated by specialist structures called transverse tubules (T-tubules), as reviewed in [1]. As vital sites for ion transport and excitation-contraction coupling, they are important to the health and functioning of cardiac cells [1]. T-tubules are more than just the structural elements of the cardiomyocyte sarcolemma; they are dynamic entities whose stability is essential to the accurate control of calcium signalling and cardiac contraction [1]. This review investigates the critical roles that T-tubules play in preserving cardiac function in healthy individuals and looks at how they change in cardiac disorders, specifically heart failure. This work highlights how disease states alter T-tubule architecture, emphasises how these changes affect heart failure, and looks into possible treatment approaches to lessen these consequences. Moreover, this review investigates the influence of physical activity on the density of T-tubules and its implications for cardiovascular well-being. The advancement of contemporary imaging technology has created opportunities for studying T-tubules in a more comprehensive manner,

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offering unprecedented volumes of data that may pave the way for innovative therapeutic approaches aimed at rectifying T-tubule abnormalities in cardiac diseases This research attempts to summarise knowledge on the crucial role of T-tubules in both health and disease by a systematic review of recent findings and existing literature, enabling a clearer awareness of their potential as targets in therapeutic interventions for cardiac disorders.

## 2. Fundamentals of Cardiac Transverse Tubules

Heart function depends on cardiac transverse tubules, which are specialised invaginations of the cardiomyocyte sarcolemma, as reviewed in Physiological Reviews (2020) [1]. These tubules, which are abundant in ion channels and carriers, aid in the regulation of action potential, support the maintenance of the resting membrane potential and allow excitation-contraction coupling [1]. From a structural perspective, t-tubules enter myocytes and create a complex network (as shown in Figure 1 anchored to sarcomeric z-discs and connected by longitudinal tubules [1]. Their phospholipid-rich membranes are maintained by the cytoskeleton and scaffolding proteins [1]. T-tubules are essential for cardiac contractility and electrophysiological processes because of this architecture, which generates microdomains that control the flow of ions and signalling molecules [1].

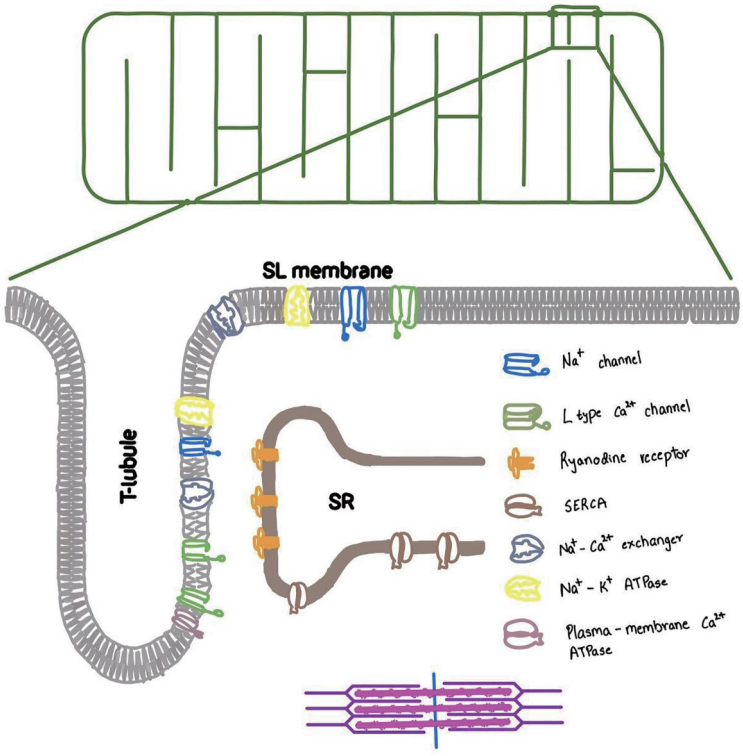


**Figure 1.** The structure of T-tubules and important proteins in the cardiomyocyte's excitation-contraction (EC) coupling. This system ensures consistent response during heart activity by means of a complex network of tubular membranes that convey electrical impulses deep into the cell, as reviewed in Setterberg et al. Ryanodine Receptors (RyRs) on the sarcoplasmic reticulum (SR), voltage-gated L-type $Ca^{2+}$ channels (LTCCs) in the T-tubules, and $Na^{+}$ channels that start cell membrane depolarization are important proteins implicated in this process. Calcium-induced calcium release occurs when LTCCs open during depolarization, allowing $Ca^{2+}$ to enter the cell and activating RyRs to release more $Ca^{2+}$ from the SR. The resulting $Ca^{2+}$ binds to the myofilaments, which causes muscle contraction. Following contraction, the sarco-endoplasmic reticulum ATPase (SERCA) facilitates the recycling of $Ca^{2+}$ ions back into the sarcoplasmic reticulum (SR), while the Na+-$Ca^{2+}$ exchanger (NCX) and the plasma-membrane $Ca^{2+}$ ATPase are responsible for removing $Ca^{2+}$ ions from the cell. The function of NCX is highly dependent on the sodium levels that are regulated by the T-tubules' $Na^{+}$ channel and $Na^{+}$-$K^{+}$ ATPase. Drawn on Notability. Abbreviations: SR, Sarcoplasmic reticulum; SL membrane, Sarcolemma membrane.

### *2.1. Structural Overview*

When transverse tubules were first identified, it was thought that the majority of t-tubules were orientated transversely across cardiomyocytes, aligned with the Z-lines [2,3]. However, it is now known that a considerable number of them also extend longitudinally, into the sarcomere's A-band [4–9]. Because of this configuration, both orientations are included under the term transverse-axial tubule system (TATS) [6, 7]. In humans or large mammals, the configuration of t-tubules within cardiomyocytes primarily consists of a transverse network while the distribution in rat cardiomyocytes is around 60% transverse and 40% longitudinal [8]. T-tubules differ in lumen diameter from 20 to 450 nm, branching irregularly, and are tortuously shaped, all of which affect its functions, as reviewed in Setterberg et al. [10]. According to one recent study, the extracellular solution is actively pumped in and out of the t-tubular compartment during the cardiac cycle, in a way that depends on the rate of contraction. This process counteracts the limitations of diffusion due to the shape of the t-tubule, a sort of functional autoregulation that was previously overlooked [11].

The structure of T-tubules varies significantly among various species and within different parts of the heart, which is a result of modifications to meet specific physiological needs [8]. T-tubules in smaller animals such as mice and rats, which exhibit faster heart rates, are notably intricate, as reviewed in Setterberg et al. [10]. These structures exhibit higher density, increased branching, and thinner walls in contrast to those observed in larger mammals such as rabbits, pigs, and humans [10]. The presence of a narrow diameter in smaller species is believed to restrict the transport of solutes within the t-tubules to a greater extent compared to larger species [12]. The t-tubule structures exhibit changes not just across different species but even within the same species, showing major differences among the chambers of the heart [10].

The t-tubule networks in both the left and right ventricles of the heart are highly organised, as reviewed in Setterberg et al. [10]. However, there is a higher degree of variability observed in the atria [10]. Studies on rats [13] and pigs [14] revealed that only a tiny percentage of atrial cells have tubular structures, despite the fact that t-tubules have been found in atrial myocytes in both small (rats) and larger mammals (pigs, dogs, sheep, cows, horses, and humans) [10]. Furthermore, when they are present, they are significantly less developed compared to the ones seen in ventricular myocytes [10]. T-tubules in atrial cells generally exhibit a lower density and tend to be oriented more longitudinally compared to ventricular cells [10].

Longitudinal tubules, or axial tubules, are predominant in atrial myocytes [15]. Axial tubules provide quick, localised calcium signalling and are less regular than transverse tubules, which are highly organised [15]. This distinction becomes crucial during atrial contraction, particularly in diseases such as atrial fibrillation, where transverse tubules are relatively few in atrial cells, while axial tubules contribute to maintaining excitation-contraction coupling [15].

As transverse tubule formations reorganise in damaged ventricles, as is the case with heart failure, longitudinal tubules may form as a compensatory mechanism [15]. Furthermore, ryanodine receptor (RyR) phosphorylation is elevated in these longitudinal tubules [15]. This phosphorylation can improve excitation-contraction coupling and calcium release, but if dysregulated, it can also lead to arrhythmias [15]. While axial tubules provide flexibility to atrial myocytes and are more important in pathological circumstances affecting the heart, transverse tubules are necessary for regular ventricular function [15].

Notably, there is a significant difference in the density of t-tubules inside the atria, with a larger concentration in the epicardium compared to the endocardium [16]. As shown in Figure 2, recent studies have indicated that there is diversity in the structure of t-tubules within the chambers of the heart [16,17]. Specifically, the density of t-tubules has been found to be lower near the apex of the left ventricle compared to other places [16,17].

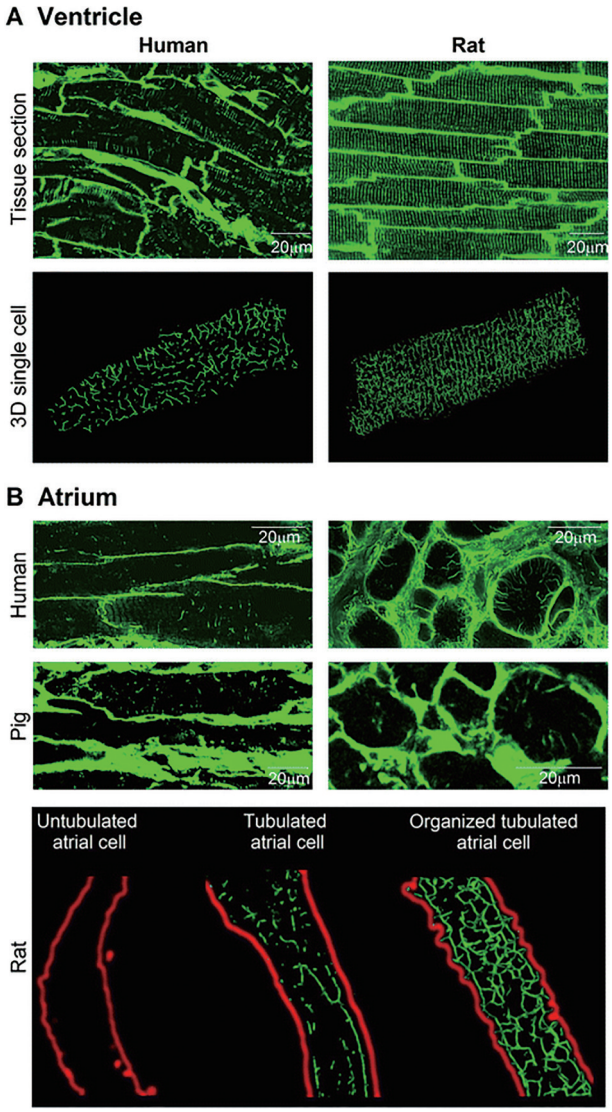


**Figure 2.** Ventricular and atrial cardiac myocytes' T-tubule organisation in humans, rats and pigs. In all three species, there was variable t-tubule organisation across the atria. Reproduced from [18].

The enormous variety in structure has a direct impact on the functioning of the heart, affecting the contractions of the heart. Understanding these distinctions is essential for formulating precise therapies for cardiac illnesses, taking into account the distinct t-tubule structure of the affected region of the heart.

### *2.2. Functional Overview*

The contraction and relaxation of cardiac myocytes, as well as the entire heart, are crucially controlled by t-tubules and their functional connections with the junctional sarcoplasmic reticulum (jSR), as reviewed by Dibb, Louch and Trafford (2022) [19]. These junctions, known as dyads or junctional membrane complexes, are stabilised by the protein junctophilin-2 (JPH2), which maintains a consistent gap width of 12–15 nm [20]. Dyads play a crucial role in facilitating the movement of $Ca^{2+}$ across both membranes during the cardiac cycle [19].

#### 2.2.1. Role in Systolic $Ca^{2+}$ Homeostasis

Cardiac myocytes undergo depolarization starting from the surface membrane and extending into the t-tubules. This depolarization triggers the activation of voltage-gated L-type $Ca^{2+}$ channels (LTCCs), as reviewed by Dibb, Louch and Trafford (2022) [19]. Consequently, there is a sudden increase in the number of

$Ca^{2+}$ ions, which then stimulates the activation of ryanodine receptors (RyRs) located in the junctional sarcoplasmic reticulum (jSR) [19]. The mechanism is known as $Ca^{2+}$-induced $Ca^{2+}$ release (CICR). The CICR mechanism plays a vital role in initiating muscle contraction by facilitating the systolic $Ca^{2+}$ transient. The arrangement and concentration of t-tubules are crucial for facilitating the synchronised release of $Ca^{2+}$ across the cell, hence impacting the velocity and uniformity of muscle contraction [19]. Smaller animals like rats and mice with elevated heart rates possess a compact and efficiently structured system of t-tubules and dyads, which guarantees a synchronised and swift $Ca^{2+}$ transient [21,22]. On the other hand, bigger mammals with slower heart rates have t-tubules that are not as tightly packed, resulting in less consistent release of $Ca^{2+}$ and a delay in contraction [23].

Atrial cells, especially those with low or no t-tubule density, exhibit a more irregular release of calcium ions, which is characterised by a U-shaped pattern as the calcium-induced calcium release (CICR) spreads inward from the surface membrane, as reviewed by Dibb, Louch and Trafford (2022) [19]. This leads to reduced speed and strength of contractions in comparison to ventricular cells. The arrangement of LTCCs and RyRs in cardiac myocytes is less organised compared to skeletal muscle, which allows for varying clustering of RyRs and the possibility of coordinated release of $Ca^{2+}$ among adjacent RyR clusters [19]. The arrangement and function of these ion channels are controlled by structural proteins such as bridge integrator 1 (BIN1), which aligns LTCCs in the t-tubule dyads [20], and junctophilin-2 (JPH2), which affects RyR activity. [19] In addition, the caveolin-3 isoform (Cav-3) that is particular to muscles helps in the creation of caveolae in the t-tubules and regulates the phosphorylation of LTCC components, thereby improving their performance [19]. The intricate interaction between structural and regulatory proteins in the dyads precisely adjusts the management of $Ca^{2+}$ in cardiac myocytes, which is essential for optimal heart performance.

#### 2.2.2. Role in Diastolic $Ca^{2+}$ Homeostasis

Following the contraction of the heart muscle, calcium ($Ca^{2+}$) is removed from the cytoplasm of the cell and stored again in the sarcoplasmic reticulum (SR) mainly through the activity of the SR $Ca^{2+}$ ATPase (SERCA), as reviewed by Dibb, Louch and Trafford (2022) [19]. Additionally, calcium is pumped out of the cell by the $Na^{+}$-$Ca^{2+}$ exchanger (NCX) and the plasmalemmal $Ca^{2+}$ ATPase (PMCA) [19]. The exchangers, which are concentrated at a higher density in the t-tubules than in the surface membrane [24, 25], play a crucial role in regulating both systolic and diastolic levels of $Ca^{2+}$. They have a significant impact on the speed and degree of cellular relaxation [19]. Elevated diastolic calcium levels can lead to a decrease in relaxation, which can interfere with the heart's ability to refill with blood [26,27].

NCX is present in both transverse and longitudinal t-tubules [28], frequently in close proximity to ryanodine receptors (RyRs), which enables the efficient elimination of $Ca^{2+}$ ions during cellular activity [29,30]. This close proximity facilitates effective calcium cycling and, under specific circumstances, can initiate increased calcium release from the ryanodine receptor (RyR) [31]. The effectiveness of the $Na^{+}/Ca^{2+}$ exchanger (NCX) is determined by local levels of sodium ions ($Na^{+}$), which are regulated by $Na^{+}$ currents and the activity of the $Na^{+}/K^{+}$-ATPase (NKA), as reviewed by Dibb, Louch and Trafford (2022) [19]. The interplay between NCX and NKA, particularly the localised modulation of $Na^{+}$ in close proximity to NCX, underscores an intricate regulatory mechanism within t-tubules that sustains cardiac function [19]. The precise coordination of cellular components in cardiac physiology is essential for the proper contraction and relaxation of the heart.

## 3. Cardiac T-Tubule Development

T-tubules are adaptable structures in the heart that adjust to fulfil physiological requirements, as reviewed by Dibb, Louch and Trafford (2022) [19]. During periods of growth, such as after birth and during physical activity, the density of t-tubules rises [32,33]. This leads to an improvement in both the speed and quantity of systolic $Ca^{2+}$ release, which in turn enhances the contractile function of the heart [19]. This modification is especially crucial in larger cardiac cells, as they cannot depend only on the release of calcium ions at the cell surface [19]. On the other hand, the process of ageing and specific cardiac conditions are linked to a decrease in t-tubule density [34], which plays a crucial role in the advancement of illnesses [35]. This decrease results in a mismatch between the density of t-tubules and the size of the cell [21]. Understanding the mechanisms that regulate t-tubule formation in healthy states could offer insights into

reversing harmful t-tubule remodelling in disease contexts.

### *3.1. Post-Natal T-Tubule Development*

T-tubules are absent in the ventricles of small mammals at birth but grow after birth [36,37]. However, in larger species such as sheep and humans, their development begins during the prenatal stage [38,39]. The variation in the timing of t-tubule development among animals may indicate distinct methods of production or differences in developmental maturity at birth, as reviewed by Dibb, Louch and Trafford (2022) [19]. Without t-tubules, the highly specialised arrangement of $Ca^{2+}$ handling machinery needed for effective $Ca^{2+}$ cycling in the adult heart is absent [19].

The formation of T-tubules affects excitation-contraction coupling by enhancing the magnitude of $Ca^{2+}$ transients and L-type $Ca^{2+}$ channel currents during the neonatal stage, as reviewed by Dibb, Louch and Trafford (2022) [19]. The interaction between these channels and ryanodine receptors (RyRs) also becomes stronger [20]. While the sarcoplasmic reticulum (SR) is not abundant at birth, RyRs are seen along the Z-line before the formation of t-tubules, and their number increases as t-tubules develop [36,40].

At the beginning, t-tubules form as small and irregular inward folds from the cell membrane [36,37,40,41], This process may be triggered by the production of caveolae, which rely on the protein Cav-3 [42, 43], as mentioned below or by endocytosis mechanisms involving the EHD1 protein [44]. The initial structures undergo extension and stabilisation through interactions that may involve vesicles, the Golgi complex, and neighbouring SR, facilitating the development of membrane components, as reviewed by Dibb, Louch and Trafford (2022) [19].

In the initial stages of development, both skeletal and cardiac t-tubules exhibit a longitudinal orientation, and RyRs are integrated into them during this period [37,45]. During the development process, these tubules align themselves transversally along the Z-line and continue to include RyRs [37,45].

### *3.2. Role of Key Proteins Involved in Post-NatalT-Tubule Development*

Several proteins, such as Cav-3, JPH2, RyR, dysferlin, and BIN1, play a role in the formation of t-tubules in the heart as shown in Figure 3 [19]. Despite the presence of compelling data highlighting their importance, investigations that include the elimination of these proteins have not completely stopped the development of t-tubules, as reviewed by Dibb, Louch and Trafford (2022) [19]. This suggests the potential existence of backup mechanisms that facilitate the growth of t-tubules, or the presence of undiscovered crucial proteins implicated in this phenomenon.

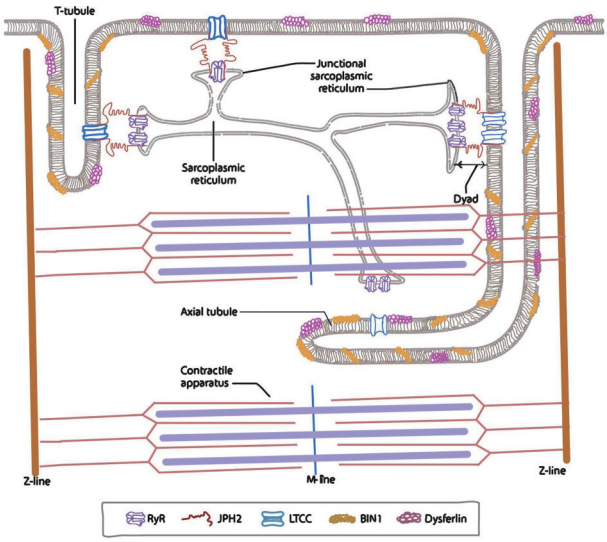


**Figure 3.** Potential regulators of t-tubule formation and maturation. This diagram illustrates the participation of essential proteins that are believed to have a role in the development of t-tubules and cardiac dyads. Adapted from [19], Drawn on Notability. Abbreviations: BIN1, bridging integrator 1; JPH2, junctophilin-2; LTCC, voltage-gated L-type $Ca^{2+}$ channel; RyR, ryanodine receptor; t-tubule, transverse tubule.

3.2.1. Cav-3

A cholesterol-binding protein called caveolin-3 (Cav-3) is essential for the development of caveolae [46]. It has also been linked to the production of t-tubules and the localization of $I_{Ca}$ and LTCC regulatory proteins to these tubules [46]. However, research has demonstrated that t-tubules can still develop, although with lower density and structural abnormalities, even when Cav-3 is eliminated in both skeletal and cardiac muscle [46,47]. The modified arrangement of skeletal muscle t-tubules in the absence of Cav-3 indicates that it has a crucial function in coordinating the formation of the growing t-tubule network [47]. In addition, the lack of caveolae in Cav-3 knockout mice suggests that other mechanisms, in addition to the production of caveolae, are also involved in the development of t-tubules [47].

3.2.2. JPH2 and RyR

The dyad-spanning JPH2 protein plays a vital role in preserving the structure of dyads and is implicated in the formation of t-tubules during cardiac maturation [48,49]. While certain research indicates an elevation in JPH2 levels during the development of the heart after birth, other studies do not show the same results [37, 48,49]. As t-tubules grow, JPH2 begins to localise at the z-disc [37]. However, if JPH2 levels are reduced, this maturation process is hindered, which in turn affects the development of transverse components [48,49]. On the other hand, elevating JPH2 expression accelerates the process of t-tubule maturation [48, 49]. The utilisation of CRISPR/Cas9-AAV9 in a study to induce mutations in JPH2 inside specific cardiac cells indicates that the development of t-tubules can occur almost normally even in the absence of JPH2 [50]. This suggests that the primary function of JPH2 may be to preserve t-tubules rather than to create them [50]. This work also emphasises the potential involvement of the ryanodine receptor (RyR) in the development of t-tubules, leading to issues about whether RyRs provide structural support to t-tubules or if the release of calcium ions in certain areas stimulates their growth [50].

3.2.3. Dysferlin

Dysferlin, a member of the Ferlin family, is a protein responsible for repairing cell membranes, as reviewed by Dibb, Louch and Trafford (2022) [19]. It has a role in the creation of t-tubules in skeletal muscle and perhaps in the heart [51]. It has the ability to localise to growing t-tubules and can also create tubule structures in cells that typically do not have them [52], indicating a potential role in t-tubule production [53]. Dysferlin deficiency results in the formation of abnormal t-tubules in skeletal muscle, which are characterised by their enlarged size, uneven shape, and alignment in a longitudinal direction [19]. Moreover, the growing coexistence of dysferlin with the L-type calcium channels (LTCC) during the development of the heart, as well as the reported restructuring of t-tubules in dysferlin knockout (KO) experiments, suggests that dysferlin may serve comparable purposes in cardiac muscle [51,54]. Dysferlin, in conjunction with other proteins such as Cav-3 and EHD1, also contributes to the process of vesicle trafficking, which is crucial for the formation of t-tubules [20]. Dysferlin is believed to facilitate the fusion of Cav-3-containing vesicles with growing t-tubules in skeletal muscle [55]. It is possible that this mechanism also takes place in cardiac muscle, although this has not been verified yet.

3.2.4. BIN1/Amphiphysin 2

BIN1, a member of the BAR (Bin-amphiphysin-Rvs167) domain superfamily, sometimes referred to as amphiphysin 2 (AmphII), plays a vital role in the formation of t-tubules in both skeletal and cardiac muscle, as reviewed by Dibb, Louch and Trafford (2022) [19]. This protein has the ability to generate t-tubule structures in cells that typically do not have them. [56–58] If this protein is completely removed, it results in mortality shortly after birth due to a heart condition called cardiomyopathy, highlighting its crucial function [56 – 58]. Although both BIN1 and dysferlin are capable of generating t-tubules, the resulting structures exhibit noticeable variations, indicating that they exert effect on various components of the t-tubule system [52]. Reducing EHD1, which is a negative regulator of BIN1's tubulating activity, causes aberrant and excessive growth of t-tubules [59,60]. In addition, the involvement of BIN1 in the process of endocytic recycling may potentially play a part in the formation of t-tubules [61]. However, the

precise contribution of BIN1 to the development of cardiac t-tubules, particularly through endocytic capture, remains uncertain.

Although BIN1 plays a crucial role, t-tubules still develop in mice with a specific deletion of BIN1 in the heart, suggesting the existence of multiple mechanisms involved in t-tubule formation [62]. BIN1 has an impact on the structure of dyads by securing microtubules that transport LTCCs to t-tubules [63]. Additionally, it has a role in attracting phosphorylated RyRs to dyads during β-adrenergic stimulation, affecting the structures of both t-tubules and SR membranes [64].

Role of BIN1 in shaping T-tubules: BIN1 has a vital function in detecting and producing membrane curvature via its N-terminal BAR domain [65]. This gene is crucial for the development of t-tubules in skeletal muscle, as reviewed by Dibb, Louch and Trafford (2022) [19]. It consists of 20 exons, which include both ubiquitous and tissue-specific ones [19]. Its phosphoinositide (PI)-binding domain plays a significant role and is necessary in T-tubule development in skeletal muscle [56,66].

The importance of the BIN1 PI domain in cardiac muscle remains a matter of discussion. Although mouse BIN1 isoforms do not have the PI domain [62], the predominant isoforms in sheep and rats possess it, which facilitates tubulogenesis in a manner similar to that observed in skeletal muscle [57,67]. This suggests that there may be variations in how BIN1 affects the structure of t-tubules, which could be particular to certain species and reliant on the isoform of BIN1.

The BIN1+13+17 isoform in mice plays a role in the formation of tightly packed folds in the inner membrane of t-tubules [62]. This may affect the movement of ions if it provides more resistance to flow than the ion channels and have an impact on the electrical activity of the heart, perhaps leading to an increased risk of arrhythmias [62]. Sheep, being larger mammals, do not have these microfolds in their hearts due to the absence of this particular isoform [57]. Recent discoveries have emphasised the overall significance of BIN1 near the Z-line in the heart, where it anchors tubule-forming isoforms that contain the PI domain [67]. These responsibilities of BIN1 may extend beyond species differences [67]. Additional investigations are required to comprehensively comprehend the diverse functions of various BIN1 isoforms in t-tubule dynamics across different species and situations.

## 4. T-Tubule Remodelling during Heart Failure

Heart failure (HF) is a major global health issue that may be categorised into two main types: HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF) as reviewed in Setterberg et al. [10]. HFrEF is characterised by the heart's impaired ability to contract, leading to inadequate delivery of oxygen to the body [10]. This syndrome is characterised by symptoms such as ventricular dilation and myocardial weakness [10]. Conversely, HFpEF is distinguished by the hypertrophy of the myocardium, which does not affect the ejection fraction [68]. However, it impairs the heart's ability to relax and fill sufficiently, leading to a reduction in cardiac output [11]. Both diseases have high fatality rates [11].

An extensive study has been conducted on HFrEF, with a specific emphasis on examining alterations in t-tubule structure and roles in different species and disease states [69]. In patients with HFrEF, there is usually a reduction in the density of T-tubules and a change in their orientation towards a more longitudinal direction, as reviewed in Setterberg et al. [10]. However, the general structure of the T-tubule framework remains intact [10]. These findings indicate that the reduction in t-tubule density may be attributed to the cell's inability to adjust to larger size, rather than a genuine loss of t-tubules [10]. Several investigations have also observed structural alterations such as the widening of t-tubules [3,70–74], the disappearance of tubule openings at the cell surface [37,73], and the development of extensive t-tubule sheets [74–76]. Comparable t-tubule abnormalities have been identified not only in the left ventricle but in the right ventricle and atrial cells as well, and they are not exclusive to patients of heart failure with reduced ejection fraction (HFrEF) [11]. Studies using disease models have demonstrated that these alterations are frequently more prominent in proximity to regions impacted by illnesses such as myocardial infarction [74, 77, 78]. The results obtained from animal disease models are in agreement with those obtained from human investigations, where samples were obtained from individuals with non-ischaemic end-stage heart failure listed for cardiac transplantation demonstrating a consistent pattern of t-tubule remodelling in cases of HFrEF. [10]

### *4.1. Impact of T-Tubule Remodelling during Heart Failure on Dyadic Structure and Systolic $Ca^{2+}$*

Studies have shown that the ventricular t-tubule network plays a vital role in regulating cardiac calcium ($Ca^{2+}$) dynamics [79, 80]. This has been demonstrated through experiments where cardiac myocytes were treated with formamide or the antidepressant imipramine to eliminate t-tubules [79, 80]. This resulted in a significant decrease in membrane capacitance and changes in the way $Ca^{2+}$ is handled [79,80]. Detubulation results in the dispersion and reduction of the systolic $Ca^{2+}$ transient, which propagates like a wave from the periphery to the core of the cell, as reviewed by Dibb, Louch and Trafford (2022) [19]. The modification reflects the same disruptions in calcium ion flow that are found in heart failure, which occur due to the disruption of the t-tubule network [19].

In cases of heart failure, the decrease or modification of t-tubules results in a process called dyadic orphaning [22]. This refers to the loss or weakening of the connection between the L-type $Ca^{2+}$ channels (LTCCs) located on the t-tubules and the sarcoplasmic reticulum (SR) $Ca^{2+}$ release channels (RyRs) [22]. The process of decoupling leads to a reduction in the magnitude of the $Ca^{2+}$ transient [20]. Although t-tubules in failing hearts are partially preserved, their ability to coordinate the release of $Ca^{2+}$ is hindered by structural alterations at the molecular level, such as modifications in the dimensions, spacing, and arrangement of RyR clusters [81]. These alterations have the ability to change the exact release of $Ca^{2+}$ during heart contraction [81].

Nevertheless, it is important to view these alterations in a broader perspective of heart failure, which encompasses a decrease in SR $Ca^{2+}$ uptake, an increase in $Ca^{2+}$ efflux, and a reduction in $Ca^{2+}$ entry through LTCCs [82,83]. The larger physiological changes mentioned here may either offset or worsen the impact of the modified t-tubule structure and function. This emphasises the intricate nature of linking changes in cardiac function to particular cellular modifications.

### *4.2. Impact of T-Tubule Remodelling during Heart Failure on β-Adrenergic Signalling*

In heart failure, the response to β-adrenergic stimulation, which is crucial for heart function, is considerably diminished [83, 84]. Studies have demonstrated that there are disturbances occurring at each step of the catecholamine signalling pathway [85]. These disruptions involve alterations in receptor quantities, responsiveness to signals, as well as different enzymes and phosphatases [85]. Consequently, these disruptions result in a diminished ability to effectively carry out phosphorylation at target sites [85]. More specifically, the remodelling of t-tubules in heart failure has been associated with these disturbances [85].

Using modern methods such as Förster resonance energy transfer (FRET) and scanning ion conductance microscopy (SICM) researchers have demonstrated that in cases of heart failure, the localization of β2-adrenergic receptors transfers from the t-tubules to other regions of the cell membrane [84]. Similarly, the routes facilitated by β3-adrenergic receptors [86] and natriuretic peptide receptors [87], which are generally localised at the t-tubules in a healthy heart, also demonstrate comparable redistributions [88].

The methodological difficulties with these investigations, however, include possible species differences and variances brought on by the environment under which the cells are cultured, as reviewed by Dibb, Louch and Trafford (2022) [19]. It is crucial to take into account these constraints, as well as the intricate interplay between various signalling pathways, particularly those involving cyclic nucleotides, which are believed to create localised complexes within cells rather than spreading freely [88].

Overall, the impact of t-tubule remodelling on heart function goes beyond basic alterations in calcium signalling and involves complex modifications in cellular signalling pathways, highlighting the t-tubule's crucial position as a major signalling hub in the heart [19]. The advancement of more accurate FRET sensors that specifically target cellular regions holds the potential to greatly improve our comprehension of these mechanisms [89].

### *4.3. Consequences for Arrhythmia Generation*

Remodelling of T-tubules in heart failure (HF), specifically in heart failure with reduced ejection fraction (HFrEF), has substantial implications for both cardiac function and the production of arrhythmias [90]. In heart failure with reduced ejection fraction, modifications in the structure of t-tubules have a role in

the disruption of calcium ($Ca^{2+}$) movement, affecting both the contraction and relaxation of the heart and increasing the likelihood of irregular heart rhythms through many mechanisms:

#### 4.3.1. RyR Leakage and Arrhythmia Triggers

Remodelling frequently results in the formation of "orphaned" RyR (ryanodine receptor) sites, where the usual strong connection with L-type $Ca^{2+}$ channels (LTCCs) is no longer present [91]. $Ca^{2+}$ sparks primarily occur at intact dyads, where there is a strong connection between t-tubules and RyRs; however, when decoupling occurs, it can result in less efficient $Ca^{2+}$ release and control [92–94]. If spontaneous $Ca^{2+}$ release occurs at these unoccupied sites, it is less likely to cause arrhythmogenic events such as early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs), which are caused by the current generated by the sodium-calcium exchanger (NCX) due to the displacement of the NCX, which impacts the removal of $Ca^{2+}$ from the cell [30,95].

#### 4.3.2. Increased $Ca^{2+}$ Influx and SR Overload

Remodelling can also cause reduced calcium-dependent inactivation of LTCCs as a result of the displacement of RyRs in space to being further away from LTCCs, which can potentially cause an increase in $Ca^{2+}$ influx [96]. This, in turn, might contribute to sarcoplasmic reticulum (SR) $Ca^{2+}$ overload, thereby further complicating the cellular $Ca^{2+}$ balance [96].

#### 4.3.3. Alterations in EAD Dynamics

Modifications in the T-tubule structure impact the arrangement of LTCCs, which may enhance the probability of EADs being initiated by the reopening of LTCCs, as reviewed in Setterberg et al. [10]. The phosphorylation status of these channels is controlled by enzymes such as calcium-calmodulin kinase II [97] and protein kinase A [98].

#### 4.3.4. Arrhythmia Risks Due to Structural Alterations

Changes in the shape of the t-tubules, such as expansion or bending, generate conditions that may affect the electrical resistance to current flow, which in turn affects the length of the action potential and raises the chances of experiencing irregular heart rhythms [62].

#### 4.3.5. Pro-Arrhythmic Alternans

T-tubule remodelling is associated with the presence of alternans, which refers to the fluctuation in the duration of cardiac action potential and the magnitude of $Ca^{2+}$ transient from one beat to another, as reviewed in Setterberg et al. [10]. This variation serves as an early indication of and may also trigger more serious arrhythmias [10]. This phenomenon is believed to happen more often in ventricular myocytes that have undergone structural changes in their t-tubules, and to a lesser degree in atrial myocytes, depending on the density of their t-tubules [99].

In summary, remodelling of t-tubules in heart failure with reduced ejection fraction (HFrEF) not only disturbs the essential calcium signalling required for heart contraction but also has a substantial impact on the occurrence and treatment of cardiac arrhythmias, as reviewed in Setterberg et al. [10]. It is imperative to conduct future research that incorporates these ideas in order to further the development of more effective treatments for heart failure.

### *4.4. Regulators of T-Tubules in Heart Failure*

As mentioned before, there has been a growing number of proteins suggested to play a role in regulating the development of t-tubules and the arrangement of important $Ca^{2+}$ regulatory proteins at the dyad. In the following section, we examine some newly identified elements that may play a role in the pathological remodelling of the t-tubule and dyadic structure in heart failure. (As shown in Figure 4).

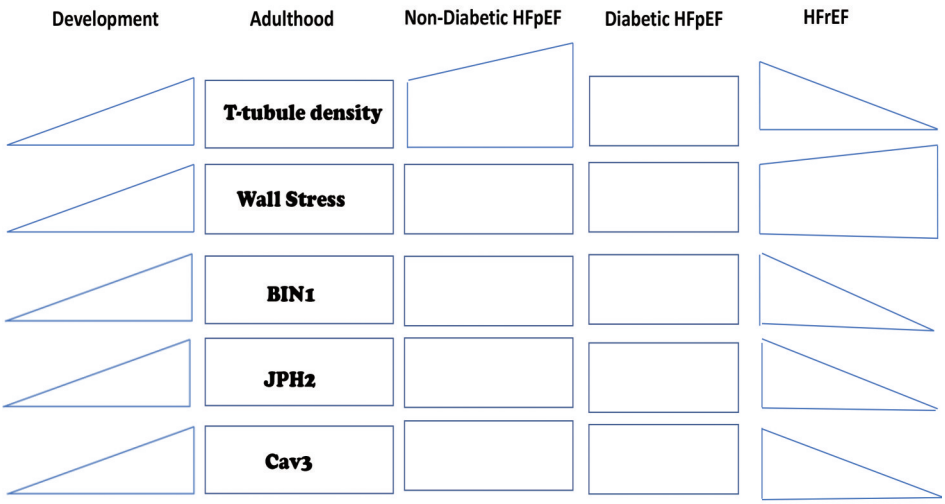


**Figure 4.** Principal regulators of t-tubule structure during development, adulthood, and heart failure caused by different factors. Adapted from [19], Drawn on Powerpoint. Abbreviations: HFpEF, Heart failure with preserved ejection fraction; HFrEF, Heart Failure with reduced ejection fraction; BIN1, bridging integrator 1; JPH2, junctophilin-2; Cav3, caveolin 3.

#### 4.4.1. Cav-3

Cav-3 is vital in the formation of t-tubules and caveolae, which are critical structures for the proper functioning of the heart [43]. Recent research emphasises that decreased expression of Cav-3 can have a major effect on cardiovascular health, especially in cases of heart failure, as reviewed in Setterberg et al. [10]. Cardiac dysfunction, characterised by reorganised t-tubules and reduced L-type calcium channel (LTCC) currents, has been seen in experiments with Cav-3 mutant mice [46]. In addition, animals subjected to pressure overload-induced cardiac stress exhibited a significant decrease in t-tubular calcium currents and calcium release [100]. The decrease was discovered to be linked to diminished levels of Cav-3. Conversely, a study involving the excessive production of Cav-3 in animals exposed to similar stressful conditions demonstrates that increased levels of Cav-3 can protect the heart from harm by maintaining t-tubular calcium currents [101]. The interaction between Cav-3 and JPH2 indicates that Cav-3 likely has a protective function in the heart. This implies that Cav-3 not only contributes to the structural stability of the heart, but also enhances its resilience to stress [102].

#### 4.4.2. JPH2

The involvement of JPH2 in heart failure is still a subject of discussion due to contradictory findings about alterations in its levels, with certain studies indicating a drop [35, 74] while others demonstrating no change [103]. These discrepancies are not directly associated with the extent of heart impairment [35, 103]. However, JPH2 may have a substantial impact on heart failure, as indicated by various hypotheses:

1. Phosphorylation mediated by SPEG: The activity of JPH2 may be influenced by phosphorylation rather than alterations in its quantity [104]. SPEG, a protein kinase that binds to JPH2 and RyR, phosphorylates JPH2, perhaps causing disruption to the t-tubule structure and calcium control in cases of heart failure [104]. SPEG also impacts calcium dynamics by altering RyR and SERCA, enzymes crucial for calcium regulation, perhaps worsening issues resulting from modified JPH2 phosphorylation, as reviewed by Dibb, Louch and Trafford (2022) [19].
2. Calpain Cleavage: JPH2 undergoes cleavage by a protein called Calpain, resulting in the formation of a fragment (JPH2-NT) that translocates to the nucleus and inhibits the function of MEF2, a transcriptional regulator associated with cardiac muscle hypertrophy and pathology [105, 106]. Inhibiting MEF2 can effectively halt or reverse hypertrophy [107]. Moreover, the use of gene therapy specifically aimed at SERCA2a can diminish this cleavage, indicating a potential approach to enhance t-tubule arrangement and cardiac performance [108].

3. Interaction with LTCC: JPH2 interacts directly with components of the LTCC, playing a vital function in aligning and coordinating calcium channels (LTCC) and RyR at the dyads [109]. The relationship between JPH2 cleavage and cardiac function is crucial, since it can have a detrimental impact on the heart muscle's reactivity and structure, particularly in stressful circumstances, as reviewed by Dibb, Louch and Trafford (2022) [19].

The analysis of JPH2's function emphasises its intricate and potentially vital role in the development of heart failure and identifies possible areas for therapeutic intervention.

#### 4.4.3. Nexilin

Recent work has identified Nexilin (NEXN) as a vital element in heart anatomy, particularly in the creation and repair of dyadic membranes [110]. Initially identified as a protein linked to actin and Z-disk structures in muscle cells, NEXN has been demonstrated to be crucial for the formation of t-tubules [110]. In a recent study, mice lacking NEXN were unable to produce t-tubules and experienced early cardiac disease and premature death [110]. Similarly, the elimination of NEXN from mature cardiac cells resulted in alterations in the structure of t-tubules, which are characteristic of heart failure with reduced ejection fraction [111]. Specifically, there was a decrease in transverse t-tubules and an increase in longitudinal ones [111].

In addition, NEXN seems to have a close functional relationship with other crucial cardiac proteins, including JPH2 and RyRs [110,111]. When NEXN is absent, it leads to a decrease in the amounts of these proteins, which in turn disrupts the balance of calcium inside the cells of the heart [110,111]. The association between NEXN and heart health is emphasised by evidence indicating that mutations in the NEXN gene are correlated with heart disease in both animals and humans [112–115]. While NEXN levels remain consistent in patients with HFrEF [116], additional investigation is required to comprehensively comprehend the role of NEXN in heart function and disease.

#### 4.4.4. Mitsugumin 53 (MG53)

MG53, often referred to as TRIM72 [117], is gaining recognition as a significant controller of t-tubules [118]. Recent study suggests that MG53 is not essential for the initial formation or regular maintenance of t-tubules, but it becomes vital during periods of stress, such as sickness or injury [119]. During such circumstances, MG53 functions as a "therapeutic agent" that migrates to impaired regions of the cellular membrane in order to close any disturbances, thereby safeguarding the integrity of the cell and t-tubules, as reviewed in [120].

One study has shown that increased levels of MG53 can improve heart function and enhance the cell's ability to repair its membrane [121]. However, these investigations did not directly examine the influence of MG53 on the structure of t-tubules. Given the acknowledged importance of maintaining the integrity of t-tubules in heart disease, it is extremely likely that MG53 plays a significant role in sustaining this structure under stressful conditions.

## 5. Opportunities for T-Tubule Targeted Therapies in Heart Failure

### *5.1. Therapies Rebuilding T-Tubules and Improving Heart Failure*

As shown in Figure 5, exploring the proteins responsible for t-tubule formation and their regulatory mechanisms, offers promising avenues for creating novel treatments for heart failure, as reviewed by Dibb, Louch and Trafford (2022) [19]. These treatments would target the structural alterations in the heart's cells that contribute to its decline [19]. Several of these prospective medicines have already exhibited efficacy in preclinical environments. In heart failure models, for example, a recent study showed that targeting BIN1 effectively stabilises the t-tubule network and improves cardiac performance [122]. A further study highlighted the function of JPH2 in preserving t-tubule integrity and demonstrated how, in preclinical conditions, upregulating its expression can slow the course of heart failure [123]. Moreover, these proteins have the potential to function as biomarkers, aiding in the identification and classification of heart disease, as demonstrated by BIN1 [124].

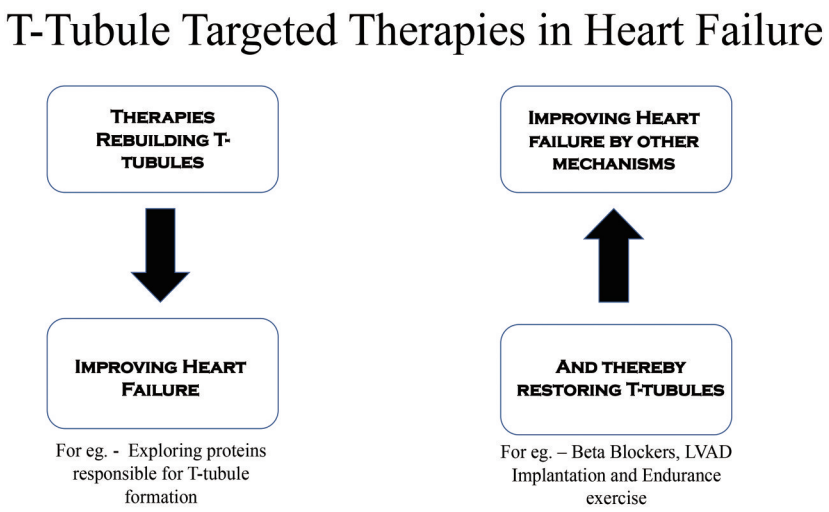


**Figure 5.** Showing the relationship between different therapies and their effect on T-tubule restoration and Heart Failure. Drawn on Powerpoint.

The relationship between t-tubules and the calcium management required for heart contractions is intricate, as reviewed by Dibb, Louch and Trafford [19]. Reestablishing the structure of t-tubules could potentially enhance calcium function and hence improve the overall functioning of the heart [19]. Cardiac failure is a gradually worsening condition in which initial changes are subtle and difficult to identify. [19] However, studies indicate that alterations in the structure of t-tubules may occur prior to the manifestation of more noticeable indications of cardiac dysfunction on an echocardiography [35]. This suggests that the remodelling of t-tubules may play a crucial role in the initial deterioration of cardiac function, emphasising its significance in both the advancement of the disease and as a focus for early intervention.

### *5.2. Therapies Improving Heart Failure (by Other Mechanisms) and Thereby Restoring T-Tubules*

Although the exploration of new proteins and pathways for future heart failure treatments shows promise, it is crucial to acknowledge that certain present medications already assist in reducing t-tubule remodelling and even restoring their density in situations of heart failure (refer to Figure 4), as reviewed by Dibb, Louch and Trafford (2022) [19]. β-blockers [125], T3-thyroxine [126], and PDE5 inhibitors [57,127] are recognised for their advantageous impact on t-tubule structure in smaller animals. These studies demonstrated that β -blockers and T3-thyroxine enhance the expression of JPH2 which, as previously discussed, is vital for maintaining the structural organization of T-tubules [125, 126]. Conversely, PDE5 inhibitors increased BIN1 levels leading to the restoration of T-tubule structure; however, the orientation was predominantly longitudinal rather than transverse [57]. In addition, mechanical interventions that relieve the heart's workload, such as cardiac resynchronization therapy on canine models [128], heterotopic transplantation in rodents [129], or left ventricular assist device (LVAD) implantation in humans [75], have demonstrated the potential to reverse t-tubule remodelling in cases of heart failure (refer to Figure 5). However, the effectiveness of these interventions may vary depending on the extent of t-tubule damage at the time of intervention [75].

Research has demonstrated that endurance exercise can improve t-tubule density after a myocardial infarction (refer to Figure 4) [33]. However, it is still unknown if exercise can prevent t-tubule loss or actively repair them, as reviewed by Dibb, Louch and Trafford (2022) [19]. The positive impacts of physical activity on t-tubules may be associated with its widely recognised capacity to decrease blood pressure and peripheral resistance, and may also be a consequence of decreasing the severity of Heart Failure, among other variables [130,131].

These findings highlight the benefits of relieving the heart from excessive strain and reducing mechanical stress, as reviewed by Dibb, Louch and Trafford (2022) [19]. Additionally, they indicate that excessive mechanical strain directly causes damage to the t-tubules, leading to heart failure [19]. This

establishes a recurring pattern in which the loss of t-tubules leads to a decrease in the strength of heart contractions [19]. Consequently, this triggers mechanisms that enhance the burden on the heart, thus exacerbating the illness [19]. Potential future treatments may aim to interrupt this cycle by specifically targeting the mechanosensory pathways that contribute to the breakdown of t-tubules in heart disease [19].

It is crucial to acknowledge that whilst different treatments can replenish the density of t-tubules in hearts impacted by heart failure, they do not consistently restore the t-tubule network to its typical organisation [57,128]. The misalignment in the t-tubule system might interfere with the connection between important components such as the ryanodine receptors (RyRs), resulting in a phenomenon called RyR orphaning, as reviewed by Dibb, Louch and Trafford (2022) [19]. This condition can lead to dyssynchronous calcium spikes, which have the potential to produce arrhythmias [22]. However, there is disagreement among researchers about this effect [132]. In order to gain a deeper understanding of and address these inconsistencies, further studies could employ sophisticated live-cell superresolution imaging microscopy techniques [19]. This technology enables researchers to view the cellular actions of calcium while simultaneously investigating the intricate structure of the t-tubule network and its components [19].

## 6. Conclusion

This report has aimed to clarify the essential function of the t-tubule network in cardiac physiology and outline the main elements that affect its regulation. We investigated the process of t-tubule remodelling in heart failure and examined prospective therapeutic targets that could improve these situations in the future. Although t-tubule remodelling plays a crucial role in the reduced contractility seen in heart failure, it is important to acknowledge that this is just one aspect of a wider range of pathological alterations. These involve modifications in various cellular structures, variations in signalling pathways, and comprehensive changes in heart metabolism and stress responses. Therefore, while t-tubule loss significantly contributes to the pathophysiology of heart failure, it is interdependent with other cellular changes, making it a critical but not an isolated target for therapeutic intervention.

To summarise, t-tubule remodelling plays a crucial role in the deterioration of cardiac function in heart failure. However, it should be understood that its effect is just one aspect of an intricate interaction involving several changes in the heart. In order to effectively treat heart failure, future therapeutic efforts should focus on maintaining the integrity of t-tubules, which are an important component of the heart's structure. This strategy should be part of a holistic treatment plan that addresses the multiple factors contributing to heart failure.

A comprehensive strategy is required that combines state-of-the-art omics technology, advanced correlative superresolution imaging, accurate genetic alterations, and creative bioengineering techniques. In order to move further, it is necessary to conduct thorough clinical trials to confirm the validity of theoretical models and laboratory findings. We acknowledge the vital need of developing a strong fundamental understanding of t-tubule dynamics. This holistic method will enhance our comprehension of cardiac biology and also facilitate the development of innovative medicines that have the potential to greatly enhance outcomes for people with heart disease.

**Funding:** This research received no external funding.

**Acknowledgments:** I would like to express my sincere gratitude to David Eisner for his invaluable guidance and insights during the preparation of this manuscript. His expertise in cardiac physiology greatly enhanced the discussions and conclusions presented in this work. I am deeply appreciative of his support and mentorship throughout this research process.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The author declares no conflict of interest.

## References

1. Hong, T.; Shaw, R. M. Cardiac T-Tubule Microanatomy and Function. *Physiol. Rev.* **2017**, *97*, 227–252. https://journals.physiology.org/doi/full/10.1152/physrev.00037.2015.

2. McNutt, N.S. Ultrastructure of the Myocardial Sarcolemma. *Circ. Res.* **1975**, *37*, 1–13. https://www.ahajournals.org/doi/abs/10.1161/01.RES.37.1.1.
3. Kostin, S.; Scholz, D.; Shimada, T.; et al. The internal and external protein scaffold of the T-tubular system in cardiomyocytes. *Cell Tissue Res. Cell Tissue Res*. **1998**, *294*, 449–460. https://link. springer. com/article/10.1007/s004410051196.
4. Simpson, F.O. The transverse tubular system in mammalian myocardial cells. *Am. J. Anat.* **1965**, *117*, 1–17. https://onlinelibrary.wiley.com/doi/10.1002/aja.1001170102.
5. Forssmann, W.G.; Girardier, L. A study of the t system in rat heart. *J. Cell Biol.* **1970**, *44*, 1–19. https://rupress.org/jcb/article/44/1/1/1663/A-STUDY-OF-THE-T-SYSTEM-IN-RAT-HEART.
6. Sperelakis, N. An orderly lattice of axial tubules which interconnect adjacent transverse tubules in guinea-pig ventricular myocardium. *J. Mol. Cell. Cardiol.* **1971**, *2*, 211–214. https://www.jmcc-online.com/article/0022-2828(71)90054-X/abstract.
7. Forbes, M. S.; Sperelakis, N. The presence of transverse and axial tubules in the ventricular myocardium of embryonic and neonatal guinea pigs. *Cell Tissue Res.* **1976**, *166*, 83–90. Available online: https://link.springer.com/article/10.1007/BF00215127.
8. Soeller, C.; Cannell, M.B. Examination of the Transverse Tubular System in Living Cardiac Rat Myocytes by 2-Photon Microscopy and Digital Image–Processing Techniques. *Circ. Res.* **1999**, *84*, 266–275. https://www.ahajournals.org/doi/10.1161/01.RES.84.3.266.
9. Pinali, C.; Bennett, H. J.; Trafford, A. W.; et al. Three-Dimensional Reconstruction of Cardiac Sarcoplasmic Reticulum Reveals a Continuous Network Linking Transverse-Tubules. *Circ. Res.* **2013**, *113*, 1219–1230. https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.113.301348.
10. Setterberg, I. E.; Le, C.; Frisk, M.; et al. The Physiology and Pathophysiology of T-Tubules in the Heart. *Front. Physiol.* **2021**, *12*, 718404. https://www. frontiersin. org/journals/physiology/articles/10.3389/fphys. 2021.718404/full#B170.
11. Rog-Zielinska, E.A.; Scardigli, M.; Peyronnet, R.; et al. Beat-by-Beat Cardiomyocyte T-Tubule Deformation Drives Tubular Content Exchange. *Circ. Res.* **2021**, *128*, 203–215. https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.120.317266.
12. Kong, C.H.T.; Rog-Zielinska, E.A.; Kohl, P.; et al. Solute movement in the t-tubule system of rabbit and mouse cardiomyocytes. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E7073–E7080.
13. Frisk, M.; Koivumäki, J.T.; Norseng, P.A.; et al. Variable t-tubule organization and $Ca^{2+}$ homeostasis across the atria. *Am. J. Physiol. -Heart Circ. Physiol*. **2014**, *307*, H609–H620. https://journals. physiology. org/doi/full/10.1152/ajpheart.00295.2014.
14. Gadeberg, H.C.; Bond, R.C.; Cherrie, H.T.; et al. Heterogeneity of T-Tubules in Pig Hearts. *PLoS ONE*. **2016**, *11*, e0156862-2. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0156862.
15. Brandenburg, S.; Pawlowitz, J.; Fakuade, F.E.; et al. Axial Tubule Junctions Activate Atrial $Ca^{2+}$ Release Across Species. *Front. Physiol.* **2018**, *9*, 1227. https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2018.01227/full.
16. Wright, P.T.; Bhogal, N.K.; Diakonov, I.; et al. Cardiomyocyte Membrane Structure and cAMP Compartmentation Produce Anatomical Variation in β2AR-cAMP Responsiveness in Murine Hearts. *Cell Rep.* **2018**, *23*, 459–469. https://www. cell. com/cell-reports/fulltext/S2211-1247(18)30398-X? _returnURL=https% 3A% 2F% 2Flinkinghub. elsevier. com%2Fretrieve%2Fpii%2FS221112471830398X%3Fshowall%3Dtrue.
17. Frisk, M.; Le, C.; Shen, X.; et al. Etiology-Dependent Impairment of Diastolic Cardiomyocyte Calcium Homeostasis in Heart Failure with Preserved Ejection Fraction. *J. Am. Coll. Cardiol.* **2021**, *77*, 405–419. https://www.sciencedirect.com/science/article/pii/S0735109720379122.
18. Manfra, O.; Frisk, M.; Louch W.E. *Regulation of Cardiomyocyte T-Tubular Structure: Opportunities for Therapy*; Springer Nature: Berlin/Heidelberg, Germany, 2017. Available online: https://www. researchgate. net/publication/316498767_Regulation_of_Cardiomyocyte_T-Tubular_Structure_Opportunities_for_Therapy (accessed on).
19. Dibb, K.M.; Louch, W.E.; Trafford A.W.Cardiac Transverse Tubules in Physiology and Heart Failure. *Annu. Rev. Physiol.* **2022**, *84*, 229–255. https://www. annualreviews. org/content/journals/10.1146/annurev-physiol-061121-040148.
20. Takeshima, H.; Komazaki, S.; Nishi, M.; et al. Junctophilins. *Mol. Cell* **2000**, *6*, 11–22. https://www.sciencedirect.com/science/article/pii/S1097276505000055.
21. Louch, W.E.; Mørk, H.K.; Sexton, J.; et al. T-tubule disorganization and reduced synchrony of $Ca^{2+}$ release in murine cardiomyocytes following myocardial infarction. *J. Physiol.* **2006**, *574*, 519–533. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1817777/.
22. Song, L.S.; Sobie, E.A.; McCulle, S.; et al. Orphaned ryanodine receptors in the failing heart. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 4305–4310.
23. Heinzel, F.R.; Bito, V.; Biesmans, L.; et al. Remodeling of T-Tubules and Reduced Synchrony of $Ca^{2+}$ Release in Myocytes from Chronically Ischemic Myocardium. *Circ. Res.* **2008**, *102*, 338–346. https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.107.160085.
24. Despa, S.; Brette, F.; Orchard, C.H.; et al. Na/Ca Exchange and Na/K-ATPase Function Are Equally Concentrated in Transverse Tubules of Rat Ventricular Myocytes. *Biophys. J.* **2003**, *85*, 3388–3396. https://www.sciencedirect.com/science/article/pii/S0006349503747584.
25. Chase, A.; Orchard, C.H. Ca efflux via the sarcolemmal Ca ATPase occurs only in the t-tubules of rat ventricular

myocytes. *J. Mol. Cell. Cardiol.* **2011**, *50*, 187 – 193. https://www. sciencedirect. com/science/article/abs/pii/S0022282810003913.
26. Ozdemir, S.; Bito, V.; Holemans, P.; et al. Pharmacological Inhibition of Na/Ca Exchange Results in Increased Cellular $Ca^{2+}$ Load Attributable to the Predominance of Forward Mode Block. *Circ. Res.* **2008**, *102*, 1398 – 1405. https://www.ahajournals.org/doi/full/10.1161/circresaha.108.173922.
27. Tanaka, H.; Namekata, I.; Takeda, K.; et al. Unique excitation–contraction characteristics of mouse myocardium as revealed by SEA0400, a specific inhibitor of Na+–$Ca^{2+}$ exchanger. *Naunyn-Schmiedeberg' S Arch. Pharmacol.* **2005**, *371*, 526–534. https://link.springer.com/article/10.1007/s00210-005-1051-9.
28. Swift, F.; Franzini-Armstrong, C.; Øyehaug, L.; et al. Extreme sarcoplasmic reticulum volume loss and compensatory T-tubule remodeling after *Serca2* knockout. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 3997–4001. https://www.pnas.org/doi/full/10.1073/pnas.1120172109.
29. Jayasinghe, I.D.; Cannell, M.B.; Soeller, C. Organization of Ryanodine Receptors, Transverse Tubules, and Sodium-Calcium Exchanger in Rat Myocytes. *Biophys. J.* **2009**, *97*, 2664 – 2673. https://www. cell. com/fulltext/S0006-3495(09)01423-4.
30. Biesmans, L.; Macquaide, N.; Heinzel, F.R.; et al. Subcellular Heterogeneity of Ryanodine Receptor Properties in Ventricular Myocytes with Low T-Tubule Density. *PLoS ONE* **2011**, *6*, e25100. https://journals. plos. org/plosone/article?id=10.1371/journal.pone.0025100.
31. Sipido, K.R.; Van Maes M. Low Efficiency of Ca2+ Entry Through the Na+-Ca2+ Exchanger as Trigger for Ca2+ Release from the Sarcoplasmic Reticulum. *Circ. Res.* **1997**, *81*, 1034 – 1044. https://www. ahajournals. org/doi/full/10.1161/01.RES.81.6.1034.
32. Haddock, P.S.; Coetzee, W.A.; Cho, E.; et al. Subcellular [$Ca^{2+}$] i Gradients During Excitation-Contraction Coupling in Newborn Rabbit Ventricular Myocytes. *Circ. Res.* **1999**, *85*, 415 – 427. https://www. ahajournals. org/doi/full/10.1161/01.res.85.5.415.
33. Kemi, O. J.; Hoydal, M. A.; MacQuaide, N.; et al. The effect of exercise training on transverse tubules in normal, *remodeled*, and reverse remodeled hearts. J. Cell. Physiol. **2011**, *226*, 2235–2243. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3096693/.
34. Kong, C.H.T.; Bryant, S.M.; Watson, J.J.; et al. The Effects of Aging on the Regulation of T-Tubular ICa by Caveolin in Mouse Ventricular Myocytes. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2017**, *73*, 711–719. https://academic.oup.com/biomedgerontology/article/73/6/711/4718153.
35. Wei, S.; Guo, A.; Chen, B.; et al. T-Tubule Remodeling During Transition from Hypertrophy to Heart Failure. *Circ. Res.* **2010**, *107*, 520–531. https://www.ahajournals.org/doi/full/10.1161/CIRCRESAHA.109.212324.
36. Ziman, A. P.; Gómez-Viquez, N. L.; Bloch, R. J.; et al. Excitation – contraction coupling changes during postnatal cardiac development. J. *Mol. Cell. Cardiol.* **2010**, *48*, 379 – 386. https://www. ncbi. nlm. nih. gov/pmc/articles/PMC3097073/.
37. Lipsett, D.B.; Frisk, M.; Aronsen, J.M.; et al. Cardiomyocyte substructure reverts to an immature phenotype during heart failure. *J. Physiol.* **2019**, *597*, 1833–1853. https://physoc.onlinelibrary.wiley.com/doi/pdf/10.1113/JP277273.
38. Munro, M.L.; Soeller, C. Early transverse tubule development begins in utero in the sheep heart. *J. Muscle Res. Cell Motil*. **2016**, *37*, 195–202. https://link.springer.com/article/10.1007/s10974-016-9462-4.
39. Kim, H. Human fetal heart development after mid-term: Morphometry and ultrastructural study. *J. Mol. Cell. Cardiol.* **1992**, *24*, 949–965. https://www.sciencedirect.com/science/article/abs/pii/002228289291862Y.
40. Seki, S. Fetal and postnatal development of $Ca^{2+}$ transients and $Ca^{2+}$ sparks in rat cardiomyocytes. *Cardiovasc. Res.* **2003**, *58*, 535–548. https://academic.oup.com/cardiovascres/article/58/3/535/298736.
41. Sedarat, F.; Xu, L.; Moore, E.D.; et al. Colocalization of dihydropyridine and ryanodine receptors in neonate rabbit heart using confocal microscopy. *Am. J. Physiol. -Heart Circ. Physiol.* **2000**, *279*, H202 – H209. https://journals.physiology.org/doi/full/10.1152/ajpheart.2000.279.1.H202.
42. Ishikawa, H. Formation of elaborate networks of t-system tubules in cultured skeletal muscle with special reference to the t-system formation. *J. Cell Biol.* **1968**, *38*, 51–66. https://rupress.org/jcb/article/38/1/51/48093/FORMATION-OF-ELABORATE-NETWORKS-OF-T-SYSTEM.
43. Parton, R. G.; Way, M.; Zorzi, N.; et al. Caveolin-3 Associates with Developing T-tubules during Muscle Differentiation. *J. Cell Biol*. **1997**, *136*, 137–154. https://rupress.org/jcb/article/136/1/137/532/Caveolin-3-Associates-with-Developing-T-tubules.
44. Hall, T.E.; Martel, N.; Ariotti, N.; et al. In vivo cell biological screening identifies an endocytic capture mechanism for T-tubule formation. *Nat. Commun.* **2020**, *11*, 3711. https://www.nature.com/articles/s41467-020-17486-w.
45. Takekura, H.; Flucher, B.E.; Franzini-Armstrong, C. Sequential Docking, Molecular Differentiation, and Positioning of T-Tubule/SR Junctions in Developing Mouse Skeletal Muscle. *Dev. Biol.* **2001**, *239*, 204 – 214. https://www.sciencedirect.com/science/article/pii/S0012160601904373.
46. Bryant, S.M.; Kong, C.H.; Watson, J.J.; et al. Caveolin-3 KO disrupts t-tubule structure and decreases t-tubular ICa density in mouse ventricular myocytes. *Am. J. Physiol. -Heart Circ. Physiol*. **2018**, *315*, H1101 – H1111. https://journals.physiology.org/doi/full/10.1152/ajpheart.00209.2018.
47. Galbiati, F.; Engelman, J. A.; Volonte, D.; et al. Caveolin-3 Null Mice Show a Loss of Caveolae, Changes in the Microdomain Distribution of the Dystrophin-Glycoprotein Complex, and T-tubule Abnormalities. *J. Biol. Chem.* **2001**, *276*, 21425–21433. https://www.jbc.org/article/S0021-9258(20)78712-0/fulltext.
48. Chen, B.; Guo, A.; Zhang, C.; et al. Critical roles of junctophilin-2 in T-tubule and excitation–contraction coupling maturation during postnatal development. *Cardiovasc. Res.* **2013**, *100*, 54 – 62. https://academic. oup. com/

cardiovascres/article/100/1/54/326137.
49. Reynolds, J. O.; Chiang, D. Y.; Wang, W.; et al. Junctophilin-2 is necessary for T-tubule maturation during mouse heart development. *Cardiovasc. Res.* **2013**, *100*, 44 – 53. https://academic. oup. com/cardiovascres/article/100/1/44/323892.
50. Guo, Y.; VanDusen, N.J.; Zhang, L.; et al. Analysis of Cardiac Myocyte Maturation Using CASAAV, a Platform for Rapid Dissection of Cardiac Myocyte Gene Function In Vivo. *Circ. Res.* **2017**, *120*, 1874 – 1888. https://www.ahajournals.org/doi/full/10.1161/circresaha.116.310283.
51. Klinge, L.; Harris, J.; Sewry, C.; et al. Dysferlin associates with the developing T-tubule system in rodent and human skeletal muscle. *Muscle Nerve* **2010**, *41*, 166–173. https://onlinelibrary.wiley.com/doi/abs/10.1002/mus.21166.
52. Hofhuis, J.; Bersch, K.; Büssenschütt, R.; et al. Dysferlin mediates membrane tubulation and links T-tubule biogenesis to muscular dystrophy. J. *Cell Sci*. **2017**, *130*, 841–852. https://journals. biologists. com/jcs/article/130/5/841/56638/Dysferlin-mediates-membrane-tubulation-and-links-T.
53. Demonbreun, A. R.; Rossi, A. E.; Alvarez, M. G.; et al. Dysferlin and Myoferlin Regulate Transverse Tubule Formation and Glycerol Sensitivity. *Am. J. Pathol*. **2014**, *184*, 248 – 259. https://www. sciencedirect. com/science/article/pii/S0002944013006640.
54. Hofhuis, J.; Bersch, K.; Wagner, S.; et al. Dysferlin links excitation – contraction coupling to structure and maintenance of the cardiac transverse–axial tubule system. *Europace* **2020**, *22*, 1119 – 1131. https://academic. oup. com/europace/article/22/7/1119/5861012.
55. Europe PMC. Europe PMC. Europepmc. org. 2016. Available online: https://europepmc. org/article/med/16550931 (accessed on).
56. Lee, E.; Marcucci, M.; Daniell, L.; et al. Amphiphysin 2 (Bin1) and T-Tubule Biogenesis in Muscle. *Science* **2002**, *297*, 1193–1196. https://www.science.org/doi/abs/10.1126/science.1071362.
57. Lawless, M.; Caldwell, J.L.; Radcliffe, E.J.; et al. Phosphodiesterase 5 inhibition improves contractile function and restores transverse tubule loss and catecholamine responsiveness in heart failure. *Sci. Rep.* **2019**, *9*, 6801. https://www.nature.com/articles/s41598-019-42592-1.
58. Muller, A.J.; Baker, J.F.; DuHadaway, J.B.; et al. Targeted Disruption of the Murine Bin1/Amphiphysin II Gene Does Not Disable Endocytosis but Results in Embryonic Cardiomyopathy with Aberrant Myofibril Formation. *Mol. Cell. Biol.* **2003**, *23*, 4295–4306. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC156129/.
59. Posey, A. D.; Swanson, K. E.; Alvarez, M. G.; et al. EHD1 mediates vesicle trafficking required for normal muscle growth and transverse tubule development. *Dev. Biol.* **2014**, *387*, 179 – 190. https://www. sciencedirect. com/science/article/pii/S0012160614000190.
60. Demonbreun, A. R.; Swanson, K. E.; Rossi, A. E.; et al. Eps 15 Homology Domain (EHD)-1 Remodels Transverse Tubules in Skeletal Muscle. *PLoS ONE* **2015**, *10*, e0136679-9. https://journals. plos. org/plosone/article?id=10.1371/journal.pone.0136679.
61. Pant, S.; Sharma, M.; Patel, K.; et al. AMPH-1/Amphiphysin/Bin1 functions with RME-1/Ehd1 in endocytic recycling. *Nat. Cell Biol*. **2009**, *11*, 1399–1410. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2788952/.
62. Hong, T.; Yang, H.; Zhang, S. S.; et al. Cardiac BIN1 folds T-tubule membrane, controlling ion flux and limiting arrhythmia. *Nat. Med.* **2014**, *20*, 624–632. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4048325/.
63. Hong, T. T.; Smyth, J. W.; Gao, D.; et al. BIN1 Localizes the L-Type Calcium Channel to Cardiac T-Tubules. *PLoS Biol*. **2010**, *8*, e1000312–2.
64. Fu, Y.; Shaw, S. A.; Naami, R.; et al. Isoproterenol Promotes Rapid Ryanodine Receptor Movement to Bridging Integrator 1 (BIN1)–Organized Dyads. *Circulation* **2016**, *133*, 388 – 397. https://www. ahajournals. org/doi/full/10.1161/circulationaha.115.018535.
65. Peter, B.J. BAR Domains as Sensors of Membrane Curvature: The Amphiphysin BAR Structure. *Science* **2004**, *303*, 495–499. https://www.science.org/doi/abs/10.1126/science.1092586.
66. Fugier, C.; Klein, A. F.; Hammer, C.; et al. Misregulated alternative splicing of BIN1 is associated with T tubule alterations and muscle weakness in myotonic dystrophy. *Nat. Med.* **2011**, *17*, 720 – 725. https://www. nature. com/articles/nm.2374.
67. Li, L.L.; Guo, Q.J.; Lou, H.Y.; et al. Nanobar Array Assay Revealed Complementary Roles of BIN1 Splice Isoforms in Cardiac T-Tubule Morphogenesis. *Nano Lett*. **2020**, *20*, 6387–6395. https://www. ncbi. nlm. nih. gov/pmc/articles/PMC8486496/.
68. Pieske, B.; Tschöpe, C.; De Boer, R. A.; et al. How to diagnose heart failure with preserved ejection fraction: The HFA–PEFF diagnostic algorithm: A consensus recommendation from the Heart Failure Association (HFA) of the European Society of Cardiology (ESC). *Eur. Heart J*. **2019**, *40*, 3297 – 3317. https://academic. oup. com/eurheartj/article/40/40/3297/5557740?login=false.
69. Jones, P.P.; MacQuaide, N.; Louch, W.E. Dyadic Plasticity in Cardiomyocytes. *Front. Physiol.* **2018**, *9*, 1773. https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2018.01773/full.
70. Maron, B.J.; Ferrans, V.J.; Roberts, W.C. Ultrastructural features of degenerated cardiac muscle cells in patients with cardiac hypertrophy. *Am. J. Pathol.* **1975**, *79*, 387–434.
71. Schaper, J.; Froede, R.; Hein, S.; et al. Impairment of the myocardial ultrastructure and changes of the cytoskeleton in dilated cardiomyopathy. *Circulation* **1991**, *83*, 504–514.
72. Kaprielian, R. R.; Stevenson, S.; Rothery, S. M.; et al. Distinct Patterns of Dystrophin Organization in Myocyte Sarcolemma and Transverse Tubules of Normal and Diseased Human Myocardium. *Circulation* **2000**, *101*, 2586–2594. https://www.ahajournals.org/doi/10.1161/01.CIR.101.22.2586.

73. Lyon, A. R.; Nikolaev, V. O.; Miragoli, M.; et al. Plasticity of Surface Structures and β 2 -Adrenergic Receptor Localization in Failing Ventricular Cardiomyocytes During Recovery from Heart Failure. *Circ. Heart Fail*. **2012**, *5*, 357–365. https://www.ahajournals.org/doi/10.1161/CIRCHEARTFAILURE.111.964692.
74. Pinali, C.; Malik, N. J.; Davenport, B.; et al. Post-Myocardial Infarction T-tubules Form Enlarged Branched Structures with Dysregulation of Junctophilin-2 and Bridging Integrator 1 (BIN-1). *J. Am. Heart Assoc. Cardiovasc. Cerebrovasc. Dis.* **2017**, *6*, e004834.
75. Seidel, T.; Navankasattusas, S.; Ahmad, A.; et al. Sheet-Like Remodeling of the Transverse Tubular System in Human Heart Failure Impairs Excitation-Contraction Coupling and Functional Recovery by Mechanical Unloading. *Circulation* **2017**, *135*, 1632–1645.
76. Fiegle, D. J.; Schöber, M.; Dittrich, S.; et al. Severe T-System Remodeling in Pediatric Viral Myocarditis. *Front. Cardiovasc. Med.* **2021**, *7*, 624776.
77. Frisk, M.; Ruud, M.; Espe, E. K.; et al. Elevated ventricular wall stress disrupts cardiomyocyte t-tubule structure and calcium homeostasis. *Cardiovasc. Res.* **2016**, *112*, 443 – 451. https://academic. oup. com/cardiovascres/article/112/1/443/2469954?login=true.
78. Wang, Y.; Chen, B.; Huang, C. K.; et al. Targeting Calpain for Heart Failure Therapy: Implications from Multiple Murine Models. *JACC Basic Transl. Sci.* **2018**, *3*, 503 – 517. https://www. jacc. org/doi/full/10.1016/j. jacbts. 2018. 05.004.
79. Brette, F.; Komukai, K.; Orchard, C. H. Validation of formamide as a detubulation agent in isolated rat cardiac cells. *Am. J. Physiol. -Heart Circ. Physiol.* **2002**, *283*, H1720 – H1728. https://journals. physiology. org/doi/full/10.1152/ajpheart.00347.2002.
80. Kawai, M.; Hussain, M.; Orchard, C. H. Excitation-contraction coupling in rat ventricular myocytes after formamide-induced detubulation. *Am. J. Physiol. -Heart Circ. Physiol.* **1999**, *277*, H603–H609. https://journals. physiology. org/doi/full/10.1152/ajpheart.1999.277.2.h603.
81. Galice, S.; Xie, Y.; Yang, Y. Size Matters: Ryanodine Receptor Cluster Size Affects Arrhythmogenic Sarcoplasmic Reticulum Calcium Release. *J. Am. Heart Assoc. Cardiovasc. Cerebrovasc. Dis.* **2018**, *7*, e008724. https://www.ahajournals.org/doi/full/10.1161/jaha.118.008724.
82. Terracciano, C. Changes in sarcolemmal Ca entry and sarcoplasmic reticulum Ca content in ventricular myocytes from patients with end-stage heart failure following myocardial recovery after combined pharmacological and ventricular assist device therapy. *Eur. Heart J.* **2003**, *24*, 1329–1339. https://academic. oup. com/eurheartj/article/24/14/1329/501780.
83. Briston, S. J.; Caldwell, J. L.; Horn, M. A.; et al. Impaired β -adrenergic responsiveness accentuates dysfunctional excitation–contraction coupling in an ovine model of tachypacing-induced heart failure. *J. Physiol. 2011*, **589**, 1367–1382. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3082097/.
84. Nikolaev, V. O.; Moshkov, A.; Lyon, A. R.; et al. β2-Adrenergic Receptor Redistribution in Heart Failure Changes cAMP Compartmentation. *Science* **2010**, *327*, 1653–1657. https://www.science.org/doi/abs/10.1126/science.1185988.
85. Najafi, A.; Sequeira, V.; Diederik, W.D.; et al. β-adrenergic receptor signalling and its functional consequences in the diseased heart. *Eur. J. Clin. Investig*. **2016**, *46*, 362–374. https://onlinelibrary.wiley.com/doi/abs/10.1111/eci.12598.
86. Schobesberger, S.; Wright, P. T.; Poulet, C.; et al. β3-Adrenoceptor redistribution impairs NO/cGMP/PDE2 signalling in failing cardiomyocytes. *eLife* **2020**, *9*, e52221. https://elifesciences.org/articles/52221.
87. Subramanian, H.; Froese, A.; Jönsson, P.; et al. Distinct submembrane localisation compartmentalises cardiac NPR1 and NPR2 signalling to cGMP. *Nat. Commun.* **2018**, *9*, 2446. https://www.nature.com/articles/s41467-018-04891-5.
88. Zaccolo, M.; Zerio, A.; Lobo, M.J. Subcellular Organization of the cAMP Signaling Pathway. *Pharmacol. Rev.* **2020**, *73*, 278–309. https://pharmrev.aspetjournals.org/content/73/1/278.abstract.
89. Surdo, N. C.; Berrera, M.; Koschinski, A.; et al. FRET biosensor uncovers cAMP nano-domains at β -adrenergic targets that dictate precise tuning of cardiac contractility. *Nat. Commun.* **2017**, *8*, 15031. https://www. nature. com/articles/ncomms15031.
90. Orchard, C. H.; Bryant, S. M.; James, A. F. Do t-tubules play a role in arrhythmogenesis in cardiac ventricular myocytes? *J. Physiol*. **2013**, *591*, 4141 – 4147. https://physoc. onlinelibrary. wiley. com/doi/10.1113/jphysiol. 2013. 254540.
91. Dridi, H.; Kushnir, A.; Zalk, R.; et al. Intracellular calcium leak in heart failure and atrial fibrillation: A unifying mechanism and therapeutic target. *Nat. Rev. Cardiol.* **2020**, *17*, 732 – 747. https://www. nature. com/articles/s41569-020-0394-8.
92. Brette, F.; Despa, S.; Bers, D.M.; et al. Spatiotemporal characteristics of SR Ca uptake and release in detubulated rat ventricular myocytes. J. *Mol. Cell. Cardiol.* **2005**, *39*, 804–812. https://www.sciencedirect.com/science/article/abs/pii/S0022282805002592.
93. Meethal, S. V.; Potter, K. T.; Redon, D.; et al. Structure – function relationships of Ca spark activity in normal and failing cardiac myocytes as revealed by flash photography. *Cell Calcium* **2007**, *41*, 123 – 134. https://www.sciencedirect.com/science/article/abs/pii/S0143416006001126.
94. Louch, W. E.; Hake, J.; Mørk, H. K.; et al. Slow $Ca^{2+}$ sparks de-synchronize $Ca^{2+}$ release in failing cardiomyocytes: Evidence for altered configuration of $Ca^{2+}$ release units? *J. Mol. Cell. Cardiol*. **2013**, *58*, 41–52. https://www.jmcc-online.com/article/S0022-2828(13)00041-2/abstract.
95. Edwards, A. G.; Louch, W. E. Species-dependent mechanisms of cardiac arrhythmia: A cellular focus. *Clin. Med. Insights Cardiol.* **2017**, *11*, 1179546816686061.
96. Shiferaw, Y.; Aistrup, G. L.; Louch, W. E.; et al. Remodeling Promotes Proarrhythmic Disruption of Calcium

Homeostasis in Failing Atrial Myocytes. *Biophys. J.* **2020**, *118*, 476–491. https://www.cell.com/biophysj/fulltext/S0006-3495(19)34397-8?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0006349519343978%3Fshowall%3Dtrue.

97. Sanchez-Alonso, J.L.; Bhargava, A.; O'Hara, T.; et al. Microdomain-Specific Modulation of L-Type Calcium Channels Leads to Triggered Ventricular Arrhythmia in Heart Failure. *Circ. Res.* **2016**, *119*, 944–955. https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.116.308698.
98. Loucks, A.D.; O'Hara, T.; Trayanova, N.A. Degradation of T-Tubular Microdomains and Altered cAMP Compartmentation Lead to Emergence of Arrhythmogenic Triggers in Heart Failure Myocytes: An in silico Study. *Front. Physiol.* **2018**, *9*, 1737. https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2018.01737/full.
99. Jiang, Y.; Tanaka, H.; Matsuyama, T.; et al. Pacing-Induced Non-Uniform $Ca^{2+}$ Dynamics in Rat Atria Revealed by Rapid-Scanning Confocal Microscopy. *Acta Histochem. Cytochem.* **2014**, *47*, 59–65. https://www.jstage.jst.go.jp/article/ahc/47/2/47_14014/_article/-char/en.
100. Bryant, S.M.; Kong, C.H.; Watson, J.J.; et al. Caveolin 3-dependent loss of t-tubular *I* Ca during hypertrophy and heart failure in mice. *Exp. Physiol.* **2018**, *103*, 652–665. https://physoc.onlinelibrary.wiley.com/doi/full/10.1113/EP086731.
101. Kong, C.H.; Bryant, S.M.; Watson, J.J.; et al. Cardiac-specific overexpression of caveolin-3 preserves t-tubular *I*Ca during heart failure in mice. *Exp. Physiol.* **2019**, *104*, 654–666. https://physoc.onlinelibrary.wiley.com/doi/full/10.1113/EP087304.
102. Minamisawa, S.; Oshikawa, J.; Takeshima, H.; et al. Junctophilin type 2 is associated with caveolin-3 and is down-regulated in the hypertrophic and dilated cardiomyopathies. *Biochem. Biophys. Res. Commun.* **2004**, *325*, 852–856. https://www.sciencedirect.com/science/article/abs/pii/S0006291X04024453
103. Caldwell, J.L.; Smith, C.E.; Taylor, R.F.; et al. Dependence of Cardiac Transverse Tubules on the BAR Domain Protein Amphiphysin II (BIN-1). *Circ. Res.* **2014**, *115*, 986–996. https://www.ahajournals.org/doi/full/10.1161/circresaha.116.303448.
104. Quick, A.P.; Wang, Q.; Philippen, L.E.; et al. SPEG (Striated Muscle Preferentially Expressed Protein Kinase) Is Essential for Cardiac Function by Regulating Junctional Membrane Complex Activity. *Circ. Res.* **2017**, *120*, 110–119. https://www.ahajournals.org/doi/full/10.1161/circresaha.116.309977
105. Guo, A.; Wang, Y.; Chen, B.; et al. E-C coupling structural protein junctophilin-2 encodes a stress-adaptive transcription regulator. *Science* **2018**, *362*, eaan3303. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6336677/.
106. Lahiri, S.K.; Quick, A.P.; Samson-Couterie, B.; et al. Nuclear localization of a novel calpain-2 mediated junctophilin-2 C-terminal cleavage peptide promotes cardiomyocyte remodeling. *Basic Res. Cardiol.* **2020**, *115*, 1–13. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10113426/.
107. Wei, J.; Joshi, S.; Speransky, S.; et al. Reversal of pathological cardiac hypertrophy via the MEF2-coregulator interface. *JCI Insight* **2017**, *2*, e91068. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5621875/.
108. Wang, S.; Zhou, Y.; Luo, Y.; et al. SERCA2a ameliorates cardiomyocyte T-tubule remodeling via the calpain/JPH2 pathway to improve cardiac function in myocardial ischemia/reperfusion mice. *Sci. Rep.* **2021**, *11*, 2037. https://www.nature.com/articles/s41598-021-81570-4.
109. Gross, P.; Johnson, J.; Romero, C.M.; et al. Interaction of the Joining Region in Junctophilin-2 With the L-Type $Ca^{2+}$ Channel Is Pivotal for Cardiac Dyad Assembly and Intracellular $Ca^{2+}$ Dynamics. *Circ. Res.* **2021**, *128*, 92–114. https://www.ahajournals.org/doi/full/10.1161/CIRCRESAHA.119.315715.
110. Liu, C.; Spinozzi, S.; Chen, J.Y.; et al. Nexilin Is a New Component of Junctional Membrane Complexes Required for Cardiac T-Tubule Formation. *Circulation* **2019**, *140*, 55–66. https://www.ahajournals.org/doi/full/10.1161/CIRCULATIONAHA.119.039751.
111. Spinozzi, S.; Liu, C.; Chen, Z.; et al. Nexilin Is Necessary for Maintaining the Transverse-Axial Tubular System in Adult Cardiomyocytes. *Circ. Heart Fail.* **2020**, *13*, e006935. https://www.ahajournals.org/doi/full/10.1161/CIRCHEARTFAILURE.120.006935.
112. Hassel, D.; Dahme, T.; Erdmann, J.; et al. Nexilin mutations destabilize cardiac Z-disks and lead to dilated cardiomyopathy. *Nat. Med.* **2009**, *15*, 1281–1288. https://www.nature.com/articles/nm.2037.
113. Wang, H.; Li, Z.; Wang, J.; et al. Mutations in NEXN, a Z-Disc Gene, Are Associated with Hypertrophic Cardiomyopathy. *Am. J. Hum. Genet.* **2010**, *87*, 687–693. https://pubmed.ncbi.nlm.nih.gov/20970104/.
114. Haas, J.; Frese, K.S.; Peil, B.; et al. Atlas of the clinical genetics of human dilated cardiomyopathy. *Eur. Heart J.* **2014**, *36*, 1123–1135.
115. Liu, C.; Spinozzi, S.; Feng, W.; et al. Homozygous G650del nexilin variant causes cardiomyopathy in mice. *JCI Insight* **2020**, *5*, e138780. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7455123/.
116. Chen, C.Y.; Caporizzo, M.A.; Bedi, K.; et al. Suppression of detyrosinated microtubules improves cardiomyocyte function in human heart failure. *Nat. Med.* **2018**, *24*, 1225–1233. https://www.nature.com/articles/s41591-018-0046-2.
117. Wang, X.; Xie, W.; Zhang, Y.; et al. Cardioprotection of Ischemia/Reperfusion Injury by Cholesterol-Dependent MG53-Mediated Membrane Repair. *Circ. Res.* **2010**, *107*, 76–83. https://www.ahajournals.org/doi/full/10.1161/CIRCRESAHA.109.215822.
118. Kitmitto, A.; Baudoin, F.; Cartwright, E.J. Cardiomyocyte damage control in heart failure and the role of the sarcolemma. *J. Muscle Res. Cell Motil.* **2019**, *40*, 319–333. https://link.springer.com/article/10.1007/s10974-019-09539-5.

119. Zhang, C.; Chen, B.; Wang, Y.; et al. MG53 is dispensable for T-tubule maturation but critical for maintaining T-tubule integrity following cardiac stress. *J. Mol. Cell. Cardiol.* **2017**, *112*, 123–130. https://www.jmcc-online.com/article/S0022-2828(17)30288-2/abstract.
120. Cooper, S.T.; McNeil, P.L. Membrane Repair: Mechanisms and Pathophysiology. *Physiol. Reviews.* **2015**, *95*, 1205–1240. https://journals.physiology.org/doi/full/10.1152/physrev.00037.2014.
121. He, B.; Tang, R.; Weisleder, N.; et al. Enhancing Muscle Membrane Repair by Gene Delivery of MG53 Ameliorates Muscular Dystrophy and Heart Failure in δ-Sarcoglycan-deficient Hamsters. *Mol. Ther.* **2012**, *20*, 727–735.
122. Liu, Y.; Zhou, K.; Li, J.; et al. In Mice Subjected to Chronic Stress, Exogenous cBIN1 Preserves Calcium-Handling Machinery and Cardiac Function. *Basic Transl. Science.* **2020**, *5*, 561–578. https://www.jacc.org/doi/full/10.1016/j.jacbts.2020.03.006.
123. Reynolds, J.O.; Quick, A.P.; Wang, Q.; et al. Junctophilin-2 gene therapy rescues heart failure by normalizing RyR2-mediated $Ca^{2+}$ release. *Int. J. Cardiol*. **2016**, *225*, 371–380. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5101129/.
124. Nikolova, A.P.; Hitzeman, T.C.; Baum, R.; et al. Association of a Novel Diagnostic Biomarker, the Plasma Cardiac Bridging Integrator 1 Score, With Heart Failure With Preserved Ejection Fraction and Cardiovascular Hospitalization. *JAMA Cardiol*. **2018**, *3*, 1206.
125. Chen, B.; Li, Y.; Jiang, S.; et al. β-Adrenergic receptor antagonists ameliorate myocyte T-tubule remodeling following myocardial infarction. *FASEB J*. **2012**, *26*, 2531 – 2537. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3360148/.
126. An, S.; Gilani, N.; Huang, Y.; et al. Adverse transverse-tubule remodeling in a rat model of heart failure is attenuated with low-dose triiodothyronine treatment. *Mol. Med.* **2019**, *25*, 1 – 14. https://link.springer.com/article/10.1186/s10020-019-0120-3.
127. Xie, Y.P.; Chen, B.; Sanders, P.; et al. Sildenafil Prevents and Reverses Transverse-Tubule Remodeling and $Ca^{2+}$ Handling Dysfunction in Right Ventricle Failure Induced by Pulmonary Artery Hypertension. *Hypertension* **2012**, *59*, 355–362.
128. Li, H.; Lichter, J.G.; Seidel, T.; et al. Cardiac Resynchronization Therapy Reduces Subcellular Heterogeneity of Ryanodine Receptors, T-Tubules, and $Ca^{2+}$ Sparks Produced by Dyssynchronous Heart Failure. *Circ. Heart Fail*. **2015**, *8*, 1105–1114.
129. Ibrahim, M.; Navaratnarajah, M.; Siedlecka, U.; et al. Mechanical unloading reverses transverse tubule remodelling and normalizes local $Ca^{2+}$-induced $Ca^{2+}$ release in a rodent model of heart failure. *Eur. J. Heart Fail*. **2012**, *14*, 571–580. https://onlinelibrary.wiley.com/doi/full/10.1093/eurjhf/hfs038?scrollTo=references.
130. Whelton, S.; Chin, A.; Xin, X.; et al. Effect of aerobic exercise on blood pressure: A meta-analysis of randomized, controlled trials. *Ann. Intern. Med.* **2002**, *136*, 493–503. https://www.ncbi.nlm.nih.gov/books/NBK69571/.
131. Turner, M.J.; Spina, R.J.; Kohrt, W.M.; et al. Effect of Endurance Exercise Training on Left Ventricular Size and Remodeling in Older Adults with Hypertension. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2000**, *55*, M245–M251. https://academic.oup.com/biomedgerontology/article/55/4/M245/2948094.
132. Dries, E.; Bito, V.; Lenaerts, I.; et al. Selective Modulation of Coupled Ryanodine Receptors During Microdomain Activation of Calcium/Calmodulin-Dependent Kinase II in the Dyadic Cleft. *Circ. Res.* **2013**, *113*, 1242–1252. https://www.ahajournals.org/doi/full/10.1161/circresaha.113.301896.