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Mechano-energetic uncoupling in hypertrophic cardiomyopathy: Pathophysiological mechanisms and therapeutic opportunities

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ABSTRACT

Hypertrophic cardiomyopathy (HCM) is a frequent inherited form of heart failure. The underlying cause of HCM is generally attributed to mutations in genes that encode for sarcomeric proteins, but the pathogenesis of the disease is also influenced by non-genetic factors, which can contribute to diastolic dysfunction and hypertrophic remodeling. Central to the pathogenesis of HCM is hypercontractility, a state that is an antecedent to several key derangements, including increased mitochondrial workload and oxidative stress. As a result, energy depletion and mechano-energetic uncoupling drive cardiac growth through signaling pathways such as ERK and/or potentially AMPK downregulation. Metabolic remodeling also occurs in HCM, characterized by decreased fatty acid oxidation and increased glucose uptake. In some instances, ketones may also feed the heart with energy and act as signaling molecules to reduce oxidative stress and hypertrophic signaling. In addition, arrhythmias are frequently triggered in HCM, resulting from the high Ca^{2+}-buffering of the myofilaments and changes in the ATP/ADP ratio. Understanding the mechanisms driving the progression of HCM is critical to the development of effective therapeutic strategies. This paper presents evidence from both experimental and clinical studies that support the role of hypercontractility and cellular energy alterations in the progression of HCM towards heart failure and sudden cardiac death.

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1. Introduction

Hypertrophic cardiomyopathy (HCM) is a frequent inherited form of heart failure, affecting 1 in every 500 individuals, that is frequently caused by mutations in genes that encode for sarcomeric proteins [1]. Thick and thin filament-encoding genes account for most cases [2]. HCM is clinically defined by left ventricle (LV) hypertrophy, particularly in the interventricular **septum**, and diastolic dysfunction, which progresses to diastolic heart failure and ventricular arrhythmias, making HCM a notable cause of sudden cardiac death, particularly in young adult people with sarcomeric gene mutations [3,4].

The relationship between HCM genotype and phenotype is complex, with a wide range of presentations ranging from **asymptomatic mutation carriers** (genotype-positive, phenotype-negative; **G+/Ph-**) to **symptomatic patients** (genotype-positive, phenotype-positive; **G+/Ph+**) who exhibit significant cardiac remodeling, including hypertrophy, cellular disarray, interstitial fibrosis, and vascular dysfunction [5,6]. Some reports identify pathogenic mutations in 63 % of HCM patients, with the other 37 % of individuals having HCM of unknown origin (genotype-negative, phenotype-positive; **G-/Ph+**) [1,7]. Increased prevalence of LV outflow tract obstruction (HOCM) is reported at rest or during exercise in genotype-negative patients [8,9]. In some rare instances, patients develop systolic heart failure, defined by a reduction in ejection fraction to <50 %, cavity dilation, and hypertrophic regression [10].

This review takes a comprehensive approach to the pathogenesis of HCM, exploring both genetic and non-genetic factors that contribute to the disease. We present a thorough examination of the most recent research on the topic, emphasizing the particular significance of hypercontractility, cellular energy disruptions, redox imbalance, and metabolic abnormalities. This review fills a critical gap in the current literature by bringing together all these key components, identifying prospective therapeutic targets, and suggesting areas where future research is needed for a deeper understanding of the underlying mechanisms and to improve HCM patient management and outcomes.

2. Heterogeneity of hypertrophic cardiomyopathy

The majority of patients with hereditary HCM (G+) have an autosomal dominant pattern of inheritance, meaning they have one mutant and one wild-type allele. However, simply carrying a heterozygous sarcomere gene mutation is not enough to fully explain the pathophysiology of HCM, as the same mutation can have varying effects on different individuals [1]. Additionally, the onset of HCM symptoms often occurs later in life, usually after the age of 20, despite the presence of sarcomeric gene alterations from birth. Even minor increases in body weight during late adolescence can contribute to the penetrance of HCM in adulthood [11], highlighting the role of non-genetic factors in disease progression. Mounting evidence suggests that sex differences play a major role in the onset and progression of HCM disease [12–15]. Females were found to be underrepresented in an HCM patient group study, indicating that females are less likely to develop symptoms or develop them later in life (e.g., on average 9 years older than males) [14]. Another study in patients with obstructive HCM found that women have a thinner interventricular septum than men [13]. Asymptomatic mutation male carriers have larger atrial and ventricular dimensions and lower fractional shortening than females [15]. Furthermore, studies in HCM animal models confirm that males and females adapt differently to exercise [16].

These findings suggest that non-genetic factors contribute to the development and progression of HCM.

3. HCM: a disease characterized by hypercontractility

The root cause of the severe cardiac remodeling observed in patients with HCM remains elusive due to the varied mechanisms by which HCM can develop. Impaired myocardial relaxation, or diastolic dysfunction, has been consistently identified in both animal [17–26] and human [27–33] studies as an early hallmark of HCM development, preceding the appearance of hypertrophy. Regardless of the underlying mutations, elevated myocardial activation at low diastolic Ca^{2+} concentrations, resulting in **hypercontractility**, is a common feature of HCM [34]. **Hypercontractility**

in HCM can have **primary** causes, such as mutations in sarcomeric proteins, or can be **secondary**, driven by protein expression changes and post-translational modifications that can directly impact contractile function (see Section 4) [35–38]. Other factors that increase contractility in HCM include the location and effects of mutated amino acids, the burden of multiple gene mutations and changes to myocardial ATP/ADP ratio as a result of an increased mitochondrial workload (see Section 6.1) [35,39–42].

4. HCM progression: a disease driven by secondary modifications

While hereditary HCM (G+) is primarily caused by autosomal dominant mutations in sarcomere genes, the contribution of **non-genetic** factors cannot be ignored in the development and progression of the disease. Understanding the role of these **secondary** factors may explain differences in disease onset and severity between asymptomatic and symptomatic patients with HCM.

4.1. Protein-dosage effect and its impact on HCM progression

Initial investigations into the specific locus of mutations suggested that mutation hotspots could be responsible for the progression of HCM, with certain mutations conferring a *benign* or *malignant* phenotype [43,44]. However, these findings were based on in vitro Ca^{2+} affinity investigations of reconstituted HCM mutant proteins in solution, which does not accurately reflect the heterozygous mutant profile of HCM presentation [45,46]. The balance between mutant and normal protein expression, referred to as **protein-dosage**, provides a more straightforward explanation for the penetrance and presentation of HCM. Cases where both alleles are affected provide evidence for the mutation-dosage impact. Homozygous or compound mutations are linked to a lower life expectancy and an early requirement for heart transplantation [47,48]. This is in line with studies showing that higher amount of heterozygous mutant protein expression associates with more severe forms of HCM [19,49,50]. Further corroboration of this concept comes from studies that have directly exchanged healthy and mutant myofilament proteins in human demembranated cardiomyocytes, which revealed that myofilament function was dependent on the degree of mutant troponin reconstituted [35,39–42]. A low level of reconstitution (as low as 14%) of HCM-causing cTnT (cardiac troponin T) mutations (cTnT-I79N, -R94C and -K280N) is sufficient to promote hypercontractility in human cardiomyocytes [41,42]. The effects of protein-dosage are additionally noticeable in HCM caused by *MYBPC3* truncating mutations, many of which result in haploinsufficiency of native protein rather than the presence of a truncated cMyBP-C (cardiac myosin-binding protein-C) [51,52]. Haploinsufficiency changes contractile performance in engineered heart tissue when cMyBP-C protein levels are equal to or lower than 73 % [53]. These studies support the notion that when the mutant protein dose exceeds a hazardous threshold or the normal protein level falls below a particular level, the myocardium becomes severely hypercontractile, with phenotypic progression of disease expected to occur (i.e., transition from asymptomatic to symptomatic HCM).

The precise mechanisms that underlie protein-dosage effects in HCM remain unclear, but alterations to the protein quality control (PQC) system may play a role [54]. The PQC system maintains a balance between protein synthesis, folding, assembly, and clearance, and alterations in this system may be influenced by various factors such as comorbidities [11] and sex differences [13–15]. The impact of PQC on protein-dosage effects in HCM however remains an area of ongoing research.

4.2. The role of post-translational modifications in HCM progression

Post-translational modifications play an essential role in the pathogenesis of HCM, evoking hypercontractility and intracellular Ca^{2+} handling, which are common features of the disease. Reduced phosphorylation of downstream effectors of the adrenergic pathway and redox protein modifications via s-glutathionylation are among the key post-translational modifiers contributing to HCM progression that have been noted to date [35–38].

4.2.1. Limited β-adrenergic activation

The regulation of contractility and intracellular Ca^{2+} handling in the heart is an important physiological process controlled by adrenergic pathway activation. Stimulation of β-adrenergic receptors by catecholamines leads to an increase in cytosolic cyclic AMP levels, resulting in the activation of the downstream protein kinase A (PKA) (Fig. 1) [55]. PKA-mediated phosphorylation of Ca^{2+} handling proteins, including phospholamban, as well as myofilament proteins cTnI (cardiac troponin I) [56,57] and cMyBP-C [58,59], modulate inotropic, lusitropic, and chronotropic responses, ultimately regulating myocardial contractility. Impairments in PKA-mediated phosphorylation of myofilament proteins have been linked to cardiomyocyte hypercontractility [17,60–63]. Studies in both animal models [64] and humans [35,40,52] with HCM have shown that hypercontractility is often associated with **decreased** phosphorylation of sarcomeric protein targets, regardless of the presence of sarcomeric protein mutations. Moreover, HCM mice demonstrate reduced responsiveness to pharmacological activation by the β-adrenergic receptor agonist isoproterenol, indicating limited capacity of β-adrenergic receptor stimulation [61,65–67]. This reduced response could be due to multiple factors, including downregulation and desensitization of β-adrenergic receptors [65], hyperactivity of β-ARK1 (β-Adrenergic Receptor Kinase 1), which uncouples β-adrenergic receptors from their downstream targets [65], and changes to cAMP and A-kinase anchoring proteins (AKAPs), which mediate PKA signaling subcellular localization [68].

4.2.2. Protein redox modifications

Reactive oxygen species can cause oxidative damage to amino acids leading to protein fragmentation, cross-links and interfering with protein function [69,70]. These modifications can also indirectly affect protein function by interfering with protein phosphorylation [71]. Redox modifications to myofilament proteins involved in cardiac contraction and Ca^{2+} handling can cause hypercontractility and diastolic dysfunction [72,73]. Cysteine residues are particularly **susceptible** to reversible redox modifications such as s-glutathionylation and protein disulfide formation, which can alter protein function [74,75]. Studies have shown that s-glutathionylation is present in high levels in the sarcomeric protein cMyBP-C in HCM models [37,38], a redox event that can additionally explain the hypercontractility and diastolic dysfunction observed. Anti-oxidant treatment strategies (see Section 9) that reduce oxidative stress, and **lower** s-glutathionylation of cMyBP-C, have been shown to improve diastolic function, reverse hypertrophy, and normalize hypercontractility [38].

In the following section, we delve into the impact of hypercontractility on cardiac energy production and cellular redox balance, and their role in driving the progression of HCM. For example, proteins involved in energy buffering, such as creatine kinase, are especially susceptible to oxidative damage, as evidenced by the formation of protein disulfides [73,76]. Furthermore, studies have consistently demonstrated elevated levels of reactive oxygen species in HCM patients [38,77–84], underscoring the importance of redox imbalance in disease pathogenesis.

5. HCM: a disease driven by myocardial energetic alterations

Hypercontractility is a hallmark of human HCM [35,36]. However, this increased contractility comes at an energetic cost during HCM progression [85,86], exerting a larger strain on the heart's energy-producing mechanisms. Studies in animals [87–89] and humans [90,91] with HCM have revealed changes in cellular metabolism and energy production prior to the appearance of hypertrophy or arrhythmias. Nuclear magnetic resonance (NMR) imaging can be used to non-invasively analyze these changes and evaluate cardiac work output. The phosphocreatine/ATP (PCr/ATP) ratio, an indication of cardiac energy recycling, is consistently **decreased** in animals [87,88,92] and humans [90] with HCM, regardless of hypertrophy, as shown by ^{31}P NMR studies. Both asymptomatic and symptomatic G+ HCM patients have **lower cardiac efficiency**, as evaluated by the ratio of oxygen consumption to external work [91]. These findings support the "**engine**

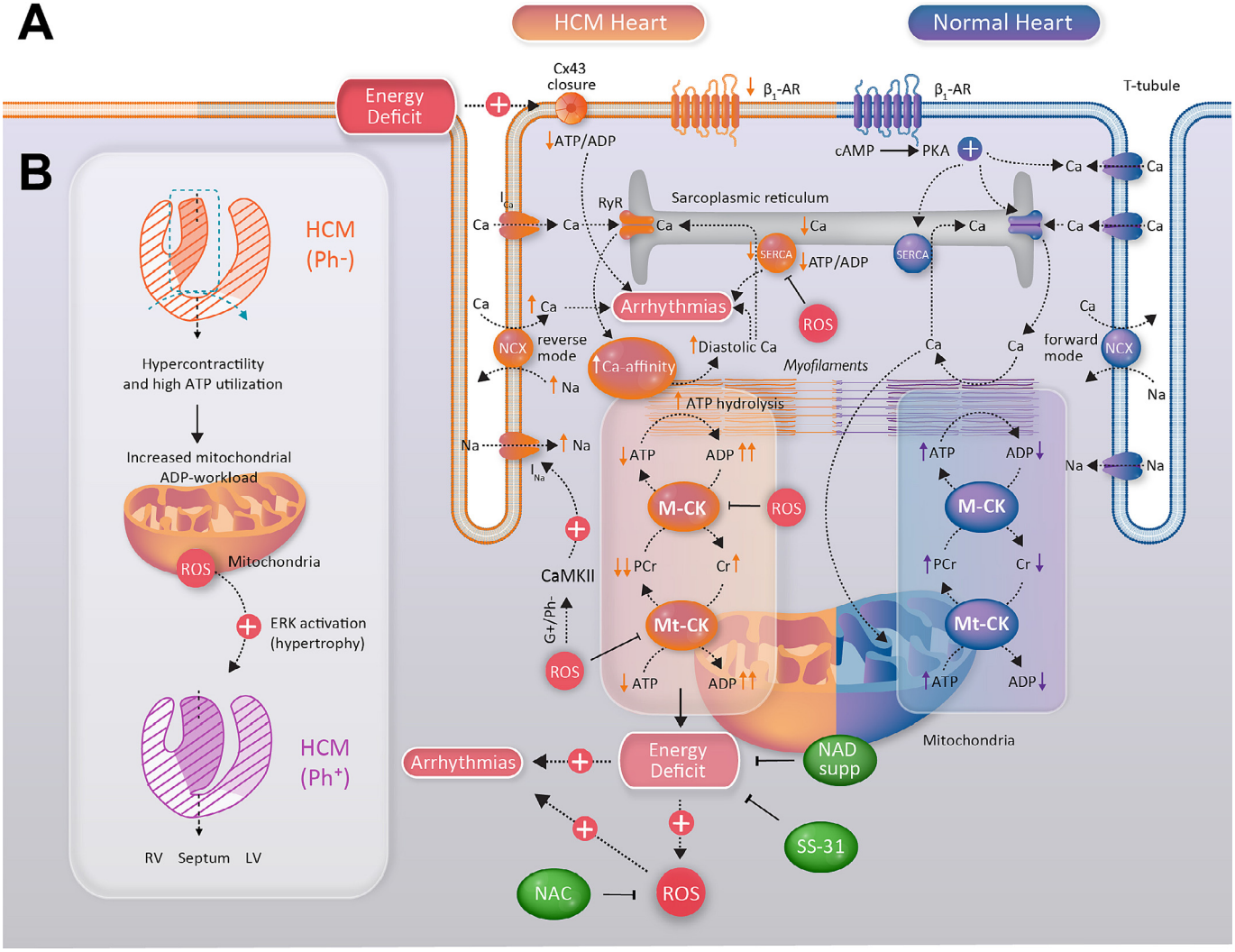


Fig. 1. Mechanisms of mechano-energetic coupling in cardiomyocytes in normal and hypertrophic cardiomyopathy (HCM), as well as its association with oxidative stress production, arrhythmia generation, and hypertrophic development. A. Normal heart, during the cardiac action potential, Ca^{2+} enters cardiomyocytes via L-type Ca^{2+} channels and triggers an even larger intracellular Ca^{2+} release from the Ca^{2+} storage organelle, the sarcoplasmic reticulum (SR). Cytosolic Ca^{2+} rises nearly tenfold from ~100 nmol/L during diastole to a peak of ~1 μmol/L in systole, activating the myofilament apparatus to contract. During diastole, Ca^{2+} diffuses away from myofilaments and is actively pumped into the SR (~70 %) by the SR Ca^{2+} ATPase (SERCA) or extruded out of the cell by Na^+/Ca^{2+} exchangers (NCX; ~30 %). The decrease of intracellular Ca^{2+} then initiates myocardial relaxation. The creatine kinase (CK)-phosphocreatine (PCr) pathway is depicted in healthy cardiomyocytes. Mitochondria phosphorylate ADP to ATP, whose phosphoryl group is used to generate PCr in an intermembrane space-localized reaction catalyzed by mitochondrial creatine kinase (Mt-CK). At the myofilaments, muscle CK (M-CK) utilizes the shuttled PCr to rapidly regenerate ATP from ADP. A. HCM heart, in cardiomyocytes from HCM patients, hypercontractility can lead to several pro-arrhythmic conditions that can result in ventricular arrhythmias, including high Ca^{2+}-buffering at the myofilaments and decreases in SERCA expression and activity. At the same time, slowed SERCA-mediated Ca^{2+} storage into the SR decelerates cytosolic Ca^{2+} decay during diastole, which is further impaired by reduced NCX-dependent Ca^{2+} extrusion, i.e., reverse mode, as a consequence of elevated intracellular Na^+ concentrations, which occurs in some of the HCM patients (G+). Cr-PCr export pathway in HCM. In conjunction with decreased Cr shuttle activity and PCr levels, impaired mitochondrial function reduces the Cr-PCr pathway's ability to buffer ATP, resulting in ADP accumulation at the myofilaments. AR, adrenergic receptor; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; SERCA, sarcoplasmic reticulum Ca^{2+} ATPase; RyR, ryanodine receptor; NCX, Na^+/Ca^{2+} exchanger; I_{Na}, Na^+ current; I_{Ca}, Ca^{2+} current; T-tubule, transversal tubule; Cx43, Connexin 43; CaMKII, Ca^{2+}/calmodulin kinase II; ROS, reactive oxygen species. B. ERK is involved in the development of cardiac hypertrophy in HCM. Activation of the redox-sensitive signaling pathway (i.e., the mitogen-activated protein (MAP) kinase ERK1/2 pathway), in response to ROS, is secondary to hypercontractility and increased mitochondrial workload. ROS can explain the close relationship between tension development (hypercontractility in HCM septum) and the degree of concentric heart growth. Lipid peroxides, such as 4-HNE (4-hydroxynonenal), may be responsible for the inhibition of AMPK activity in human HCM. Inactivation of AMPK promotes protein synthesis and the development of hypertrophy and may be involved in hypertrophy development in HCM. AMP-activated protein kinase, AMPK; NAC, *N*-acetylcysteine; SS-31, Szeto-Schiller peptide; NAD suppl, NAD supplementation. See text for details.

out of fuel" hypothesis, which proposes that the development of HCM may be driven by hypercontractility and excessive consumption of ATP, among other factors [93]. Severe decreases in the PCr/ATP ratio have been linked to cardiac decompensation in dilated cardiomyopathy, where low PCr/ATP is a prognostic sign for mortality [94]. This implies that significant and long-term changes in energy buffering mechanisms are likely to account for HCM patients who develop systolic heart failure. As we will explore further, multiple factors in HCM disrupt the regulation of cellular energy buffering mechanisms, such as creatine kinase (CK) and the mitochondria, along with rapid changes in energy utilization. Some of these modifications are likely caused by hypercontractility and excessive ATP use, which adds to the mitochondrial energetic burden.

6. Disrupted energy recycling in HCM

6.1. Disrupted CK/PCr system

The balance of energy generation and transmission is disturbed in HCM, resulting in a shift in the energy usage between the myofilaments and the mitochondria. The heart relies on the CK/PCr system and mitochondrial coupling to rapidly renew ATP at sites of energy consumption (Fig. 1) [95].

**In the intermembrane space of mitochondria, the mitochondrial isoform of creatine kinase (Mt-CK) transfers the phosphoryl group of ATP to creatine (Cr), forming PCr. The muscle CK isoform (M-CK) located at the myofilament M-band area facilitates the reverse reaction, thus regenerating ATP in close proximity to the site of utilization, while lowering [ADP]. The PCr/Cr shuttle functions as an energy carrier buffer that maintains the [ATP]/[ADP] ratio by preventing the rise in ADP levels, maximizing against the lower cellular diffusions of ATP and ADP. Even though the ATP cellular pool (about 10 mM) is smaller than the PCr pool (about 20 mM), [ATP] remains constant due to the continuous replenishment from the larger pool of PCr.*

Investigations utilizing ^{31}P NMR revealed that the PCr/ATP ratio is **lower** in both animals [87,88,92] and humans [90] with HCM, and this is accompanied by a **decrease** in **CK flux** in humans [96,97], suggesting a disruption in the PCr/CK shuttle system. Experimental studies have demonstrated that measurements of ATP levels in HCM animal models with MyHC-R403Q, cTnT-R92L, and cTnT-R92W mutations remain largely unchanged (10 mM in controls to 7 mM in HCM) even under conditions of increased cardiac workload or stress [87,88,92]. This is accompanied by significant decreases in the PCr content, from ~20 mM in controls to ~14 mM in HCM animals [87,88,92]. Notably, ADP levels can increase by >500 % up to 130 μM in HCM models, from a baseline of approximately 20 μM [87,88,92]. These changes in the ATP/ADP ratio, caused by ADP accumulation, can exacerbate the already higher contractility observed in HCM [35]. Literature on cellular expression levels and CK activity in HCM is limited. To address this, we conducted experiments using two HCM mouse models - mutant *Tnnt2*-I79N [98] and *Mybpc3*-KI [99] - aged between 12 and 16 weeks. The *Mybpc3*-KI mouse model carries the human c.772G > A *MYBPC3* mutation in the homozygous state [99] and replicates the main features of HCM, i.e., Ca^{2+} sensitization and diastolic dysfunction [24,37], while *Tnnt2*-I79N transgenic mice express the human I79N *TNNT2* mutation and exhibit high myofilament Ca^{2+} sensitivity and diastolic dysfunction, as well as susceptibility to ventricular arrhythmias during β-adrenergic stimulation [17,89,100,101]. Our findings support the idea of a disrupted PCr/CK shuttle system, as we observed decreased CK expression and activity in both HCM mouse models (Fig. 2). This inefficiency is further compounded by increased myosin-ATPase activity [85,86] and cellular hypertrophy [35,36], which puts additional strain on the PCr/CK shuttle capacity in human HCM. Despite the evidence, the mechanisms underlying the decrease in CK flow in HCM remain unknown, and it is unclear whether CK protein levels and activity are altered in human HCM myocardium.

6.2. Disrupted mitochondrial function

In both animals [23,102] and humans [103,104] with HCM, there are indications of changes in cardiomyocyte-mitochondrial architecture, function, and metabolic pathways. Mitochondrial function is determined by several factors, including morphology, number, size, localization across the myofilament space, substrate supply, enzyme activity, supercomplex quantity, and oxygen-dependent respiration. HCM models have revealed diverse ultrastructural findings relating to mitochondria, such as disorganization and loss along the myofilament space, decreased respiration, and hypoactivity of Krebs cycle enzymes [23,102]. Similarly, human HCM also shows large mitochondria with disorganized cristae and farther separation from the nearest myofibrils [103,104]. Improper spatial organization of mitochondria closely associates with limited mitochondrial respiration in human HCM, and increased septum mass in G-/Ph+, but not in G+/Ph+ HCM patients [104].

Literature on the state of mitochondrial supercomplexes in animals and humans with HCM is however limited. To address this gap, we conducted protein biochemistry experiments in our two HCM mouse models, i.e., mutant *Tnnt2*-I79N and *Mybpc3*. By targeting the five mitochondrial complexes with selective antibodies, we were able to reveal that the mitochondrial supercomplexes remain unaltered (Fig. 3). The absence of observable changes via protein immunoblotting, on the other hand, does not rule out intrinsic changes in the activities of certain mitochondrial complexes or proper respiratory chain operation, which are not evaluated by the experimental conditions. In addition, it is also likely that the observed results represent the early phases of the disease in animals that are either too young or do not have the influence of comorbidities such as hypertension, obesity, and diabetes, as is expected in human HCM [1]. Indeed, alteration of mitochondrial respiratory components in human HCM is substantiated by the fact that ex vivo treatment with NAD^+ supplementation, which enhances the availability of Krebs-cycle NADH, improves mitochondrial respiration. Furthermore, the application of a tetrapeptide compound, such as **elamipretide**, that enhances the cohesiveness of mitochondrial respirasome complexes was shown to increase the expression of complex I in human HCM (see Sections 8.1.2 and 9.2) [104].

Disrupted energy balance not only affects the heart's ability to contract and relax properly in HCM [35,105] but also has significant implications for the development of hypertrophy and the occurrence of arrhythmias, i.e., **mechano-energetic uncoupling**.

7. Mechano-energetic uncoupling in HCM

In a healthy heart, the processes of excitation-contraction coupling are closely linked to ATP synthesis in mitochondria, i.e., **mechano-energetic coupling**. During increased cardiac workload, the amplitude of cytosolic Ca^{2+} transients also increase, leading to an increase in ATP consumption and, subsequently, an acceleration of ADP delivery to the mitochondria via the creatine shuttle (Fig. 1). At the same time, Ca^{2+} accumulates in the mitochondrial matrix, which stimulates Krebs cycle dehydrogenases to regenerate NADH, thereby matching the increase in ATP demand with an increase in ATP production at the respiratory chain [106]. However, in the case of HCM myocardium, which is sensitized to Ca^{2+} at the myofilament space [35,36], the ATP demand for any given cytosolic Ca^{2+} concentration is higher than in a healthy heart [86]. Nevertheless, it is considered that the increased NADH oxidation in the respiratory chain is not accompanied by adequate Ca^{2+}-dependent stimulation of the Krebs cycle, resulting in net oxidation of the mitochondrial pyridine nucleotide pool [107]. This **bioenergetic mismatch** can further exacerbate ADP increases, which can accentuate the elevated cardiac workload seen in HCM [35,105]. Additionally, NADH oxidation reverses the reaction of the mitochondrial nicotinamide nucleotide transhydrogenase (NNT), which converts NADPH to NADH and ATP regeneration while decreasing mitochondrial anti-oxidative capacity [107]. As a result, there is an overflow of hydrogen peroxide (H_2O_2) in the mitochondria, and oxidative stress is frequently reported in HCM [38,77–84] and a well-known cause of LV hypertrophy and fibrosis in HCM models [108,109].

In the next subsection, we will further examine how the rise in **oxidative stress** in HCM can contribute to the development of **hypertrophy**.

7.1. Hypertrophy development

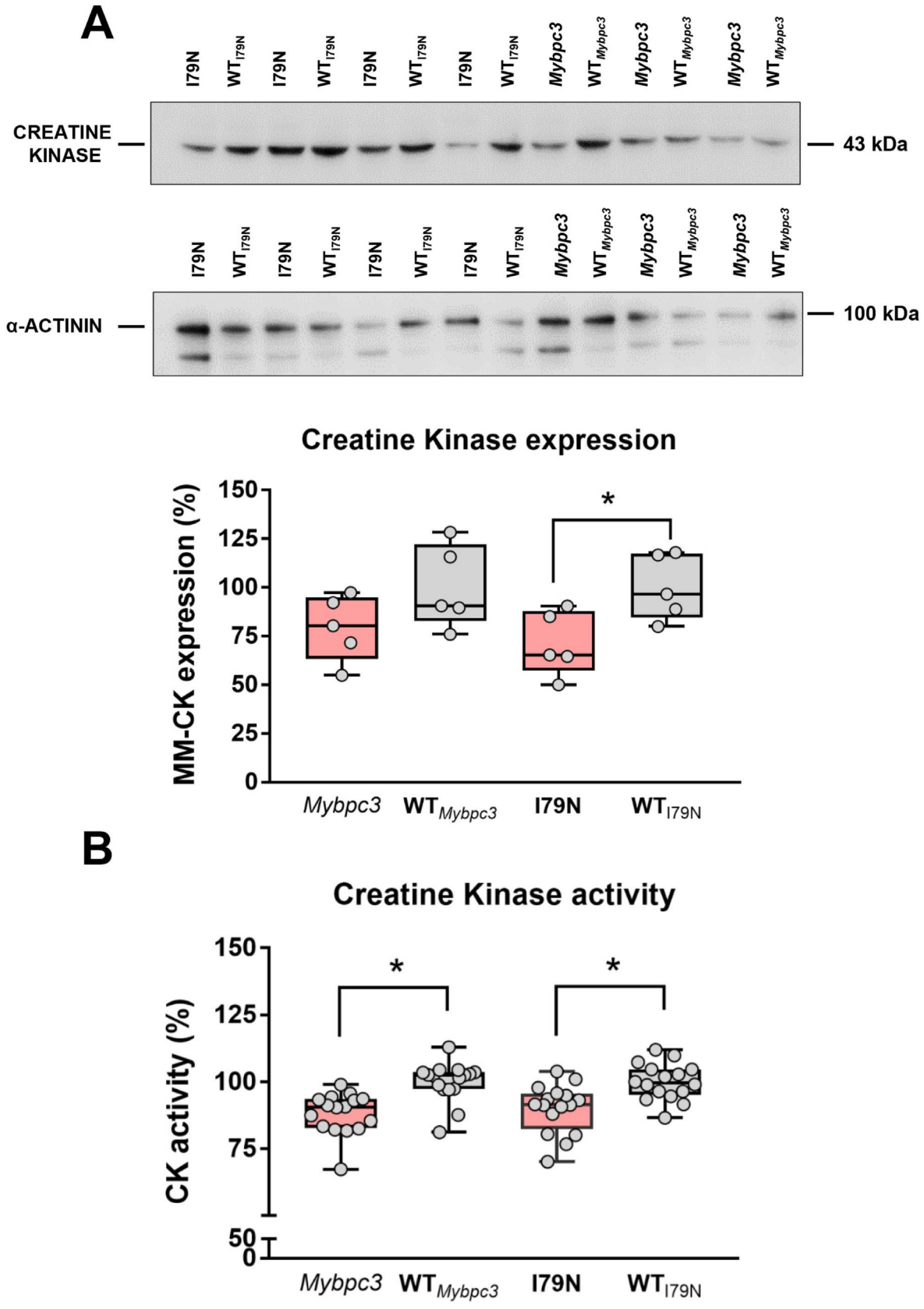
7.1.1. ROS-induced CaMKII activation

Ca^{2+}/calmodulin kinase II (CaMKII) is a pro-hypertrophic signaling pathway activated by ROS in pressure-overload hypertrophy [110,111]. ROS has been shown to oxidize histone deacetylase 4 (HDAC4) in heart failure models, causing its nuclear export and activation of the nuclear factor of activated T cells (NFAT) and the myocyte enhancer factor-2 (MEF2), leading to transcription of pro-hypertrophic genes [112]. Studies on human HCM biopsies have shown that constitutively activated CaMKII is increased exclusively in sarcomeric G+ HCM, but not in G- HCM [113]. Despite this, HDAC4, the downstream target of CaMKII, was found to be elevated in both

symptomatic G + and G- HCM genotype groups. These findings suggest that HDAC4 phosphorylation increased **independently** of CaMKII, indicating that there is no common link between ROS-induced CaMKII activation and MEF2-mediated hypertrophic signaling in human HCM [113].

7.1.2. ROS-induced ERK1/2 signaling activation

External factors such as growth factors, cytokines, and physical stress activate the mitogen-activated protein kinase (MAPK) family member, ERK1/2 pathway [38]. ROS can also activate this pathway [114], which



has been linked to HCM development [115]. When activated, ERK moves into the nucleus and modifies gene expression through phosphorylation of transcription factors, ultimately influencing the type of cardiac remodeling that occurs [115]. Hypercontractility in HCM is predicted to promote the development of concentric LV hypertrophy through activation of the MAPK/ERK pathway in a ROS-dependent manner (Fig. 1B) [115]. These findings establish a mechanism through which hypercontractility in HCM might contribute to increased mitochondrial workload and oxidative stress, inducing hypertrophic remodeling.

7.1.3. AMPK activation

Patients with HCM have symptoms that are comparable to other cardiomyopathies, such as Protein Kinase AMP-Activated Non-Catalytic Subunit Gamma 2 (PRKAG2) syndrome [116,117] and Glycogen Storage Disease Type IIIa (GSDIIIa) [118]. In these cardiomyopathies, the energy sensor AMP-activated protein kinase (AMPK) is often hypoactive and associates with substantial metabolic alterations such as activation of anabolic pathways, including protein synthesis [119,120] and glycogen storage [121,122], both of which are required for cardiac growth. In line with this, glycogen deposits was an early observation in symptomatic HCM patients [123–125] and recent proteomic analysis revealed **lower AMPK** expression in both symptomatic G+ and G- HCM [126]. One may argue for AMPK involvement in the development of cardiac hypertrophy in HCM based on the causal relationship between AMPK inhibition and increased glycogen storage and protein synthesis (Fig. 1B). Overexpression of AMPK reverses myocardial hypertrophy in pressure-overload animals [127]. The specific cause of AMPK downregulation in HCM is unknown, but higher 4-HNE levels are reported in HCM patients [84] and have been shown to exert an inhibitory action on AMPK [128]. 4-HNE is a lipid peroxidation product that is mostly derived from mitochondrial cardiolipin peroxidation (see Section 9.2.1).

In the following section, we will explore how hypercontractility, mechano-energetic uncoupling and ROS serve as a substrate for cellular **arrhythmias** in HCM.

7.2. Arrhythmogenicity

7.2.1. Hypercontractility and energy depletion: a substrate for arrhythmias in HCM

In HCM, hypercontractility can lead to various conditions that contribute to ventricular arrhythmias. These conditions involve high diastolic Ca^{2+} levels and energy depletion, which are frequent in both animals [17,87,88,92,129] and humans [96,97,130] with HCM (Fig. 1). The high diastolic Ca^{2+} levels can arise as a result of direct Ca^{2+}-buffering at the myofilaments, which can cause arrhythmias and abrupt cardiac arrest, as seen in HCM animals [17,89,129]. Additionally, a **decrease** in the ATP/ADP ratio can limit the performance of ATP-dependent cardiomyocyte ion pumps, such as the sarcoplasmic reticulum calcium-ATPase (SERCA) (Fig. 1) [131]. This slows cardiac relaxation and provides a substrate for arrhythmias in HCM by limiting Ca^{2+} removal from the cytosol. SERCA hypoactivity and low expression have been found in human symptomatic G+ and G- HCM [113,130]. Furthermore, the **reduction** in the ATP/ADP ratio can also lead to regional conduction velocity anomalies by impairing connexin 43 function at membrane junction locations [89]. This reduction can cause a decrease in the function of connexin 43, which is critical for conduction properties, and its loss of function can result in gap junction closure and regional conduction velocity reduction, providing a substrate for arrhythmias (Fig. 1) [132]. Indeed, ischemia is accompanied by gap junction closure [133], which is most likely induced by ATP depletion [134]. Gap junction uncoupling causes regional conduction velocity slowing and arrhythmias in HCM mice, which are related to diminished function of connexin 43 [89].

7.2.2. ROS-Induced CaMKII Activation in Genotype-positive (G+) HCM

In HCM, the relationship between ROS-induced activation of CaMKII and its contribution to the development of arrhythmias is a complex and multifactorial one [135]. Studies show that ROS-induced activation of CaMKII can result in the phosphorylation of Na^+ channels, resulting in an increase in the late Na^+ current ($I_{Na,L}$), intracellular [Na^+], and action potential prolongation [136]. This activation of CaMKII can also cause early and delayed afterdepolarizations (EADs and DAPDs, respectively; DADs) through the activation of L-type Ca^{2+} channel currents ($I_{Ca,L}$). In humans with HCM, CaMKII activation by ROS leads to an increase in $I_{Na,L}$ and directly contributes to action potential prolongation [137]. High diastolic Ca^{2+}, prolongation of action potential, and DADs are common observations in HCM animals [17,129] with constitutively active CaMKII [138] (Fig. 1). Notably, and as stated previously, studies in humans showed that the increases in constitutively activated CaMKII are **unique** to symptomatic G+ HCM [113], implicating differential Ca^{2+}-sensing post-translational modulation between HCM genotypes. These findings suggest that the role of CaMKII in HCM-related arrhythmias is not universal and may differ depending on the specific genotype of the disease.

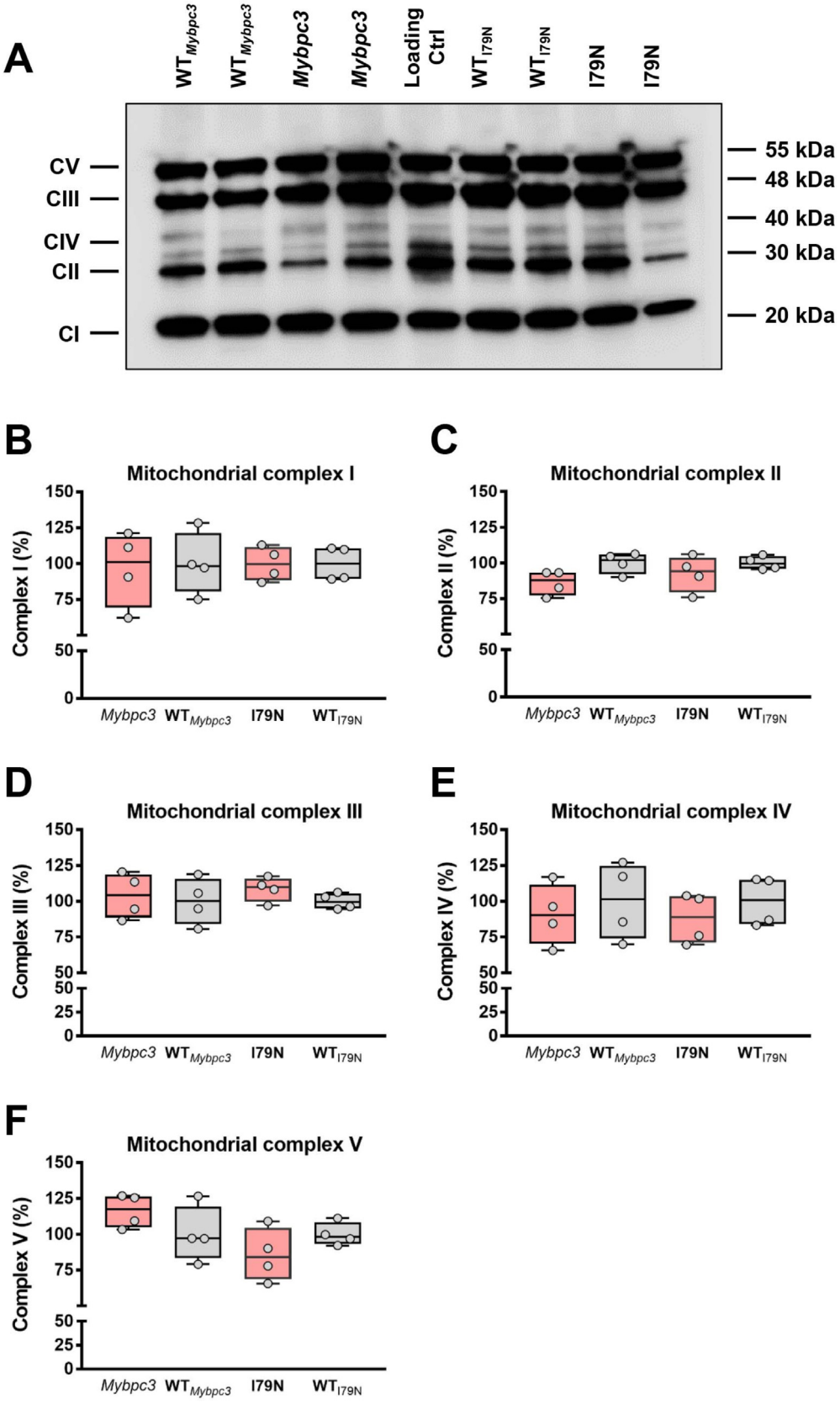
7.2.3. ROS-mediated mechanisms of arrhythmogenicity

Other mechanisms also exist by which ROS can contribute to arrhythmias in HCM. One such mechanism is the impact of ROS on diastolic Ca^{2+} levels. ROS can increase diastolic Ca^{2+} by suppressing the activity of SERCA [139,140] and oxidizing type 2 ryanodine receptors (RyR2) [141,142]. These alterations can activate the sarcolemmal Na^+/Ca^{2+} exchanger and Ca^{2+}-induced Ca^{2+} release from the SR, and result in abnormal action potentials and arrhythmias (Fig. 1). Additionally, even small amounts of mitochondrial ROS can trigger spontaneous SR Ca^{2+} release events (so-called Ca^{2+} sparks and waves), which can lead to arrhythmias [142,143]. Studies in HCM animals have shown that drug therapy aimed at reducing ROS levels can **prevent** arrhythmia episodes and sudden death (see Section 9.2.1, elamipretide discussion) [144]. Furthermore, it has been observed that hypercontractility inducing drugs can increase spontaneous SR Ca^{2+} release events and Ca^{2+} waves, which are well-defined arrhythmia triggers, but inhibition of mitochondrial H_2O_2 can prevent their occurrence (Fig. 1) [144]. In human HCM, arrhythmias may still occur even when RyR2 dysfunction by oxidation is absent, as indicated by the lack of altered RyR2 oxidation (also phosphorylation and nitrosylation) in symptomatic G+ and G- HCM [113]. This could be due to rapid cellular redox fluctuations that happen within the cell but are not detectable by protein immunoblotting techniques. Alternatively, other mechanisms such as those described in Section 7.2.1 may be more critical determinants for arrhythmias, including high diastolic Ca^{2+} levels and the reduction in ATP/ADP ratio.

Fig. 2. Profile of myofilament creatine kinase (MM-CK) in mutant *Tnnt2*-I79N and *Mybpc3* hypertrophic cardiomyopathy (HCM) mouse hearts. Mice that carry the human c.772G>A *MYBPC3* mutation at the homozygous state (*Mybpc3*-KI) and WT controls were generated in a Black Swiss background as described previously (Vignier, Schlossarek et al. 2009) and used at an age of 12–16 weeks. Transgenic mice expressing mutant or the WT troponin T (*Tnnt2*-I79N and -WT) were generated as described previously (Miller, Szczesna et al. 2001) in a mixed genetic background and used at an age of 12–16 weeks. A. Western blot with a creatine kinase (CK) specific antibody (Santa Cruz, sc-15,164) and an antibody against α-actinin (Sigma, A7811) to correct for loading following methods described in Sequeira and colleagues (Sequeira, Najafi et al. 2015). The CK antibody was diluted to a final working concentration of 2.0 μg/mL by a 500× dilution of the stock solution (1 mg/mL), while the α-actinin antibody was diluted to a final working concentration of 0.4 μg/mL by a 2500× dilution of the stock solution (1 mg/mL). B. Creatine kinase activity was evaluated at 37 °C using a colorimetric test kit (Abcam, ab65339), with signal saturation after 30 min. An unpaired *t*-test was used to compare I79N or *Mybpc3* to their respective wild-type controls. The significance level was set to $^*p < 0.05$. The data is presented as mean ± SEM and is normalized relative to controls (set to 100). A total of $N = 5$ mouse hearts with three to four intrinsic replicates (total of $n = 16$ replicates, where n is experimental repetitions) were used per each sample group.

8. Metabolic adaptation in the heart: balancing substrate usage against energy production

The unique metabolic demands of the heart make it crucial for the balance between substrate uptake and local usage to be tightly regulated. In normal conditions, fatty acid oxidation is the primary source of ATP regeneration in the heart (70–80 %), while 20 to 30 % is driven by glucose and lactate oxidation, and a smaller contribution is attributed to ketones and amino acids [145,146]. The utilization of different **substrate sources** for energy production in the heart results in variable amounts of NADH (and



$FADH_2$) generated per carbon cycle, as well as the utilization of ejected electrons and protons entering respiration. Specifically, electrons from NADH generated by glycolysis enter the mitochondria via complex I, as opposed to those obtained from fatty acid β-oxidation, which are transferred primarily via the complex electron transferring flavoprotein (CETF) and complex III [147]. β-oxidation provides a lower ATP/O_2 ratio output (but also lower H^+/O_2) than glucose; that is, more oxygen is utilized to regenerate ATP with estimated values of ~2.5 ATP/O_2 for fatty acids vs 3.17 ATP/O_2 for glucose [148].

Consequently, it is considered that after periods of elevated cardiac workload and/or myocardial remodeling, regulation of enzymes and transporters involved in metabolic pathways, and changes in coronary flow and reserve, oxygen availability may occur. These changes can promote a **shift** towards glucose-driven oxidation, reducing reliance on mitochondrial oxygen, while promoting ATP production [149,150]. This metabolic adaptation is especially noticeable in models of pressure-overload induced hypertrophy and heart failure [151–153], as it enables the heart to maintain adequate levels of ATP even in the presence of limited oxygen supply. In this section, we explore potential mechanisms underlying the metabolic adaptation in HCM progression.

8.1. Metabolic adaptability in HCM

8.1.1. Metabolic adaptability in genotype-positive (G+) HCM

The pathological mechanisms underpinning the **metabolic shift** in HCM, as well as the magnitude of changes observed, in contrast to other diseases have not been established. However, clinical [91,154] and preclinical [126] studies support that the metabolic shift is more visible in symptomatic HCM. Indeed, enhanced glucose uptake [154] and decreased myocardial oxygen consumption in symptomatic HCM patients is observed, while this is absent in asymptomatic HCM patients [91]. The precise cause of this metabolic shift remains unknown, but it is likely a complex interplay between multiple factors. Downregulation of fatty acid metabolic pathways may play a role, as demonstrated by studies showing decreased expression of proteins involved in fatty acid oxidation in symptomatic HCM biopsies [126]. Microvascular dysfunction, which is frequently observed in HCM, can reduce oxygen availability [155–158], and may also contribute to this metabolic shift. Furthermore, the high cardiac workload and oxygen consumption during the transition from asymptomatic to manifested HCM may create a mismatch between energy supply and demand, prompting the heart to rely more heavily on glucose oxidation to generate ATP [91]. Evidence of altered fatty acid pathways is further supported by studies showing decreased expression of fatty acid receptors, including cluster differentiation 36 (CD36), in symptomatic HCM but not in asymptomatic cases [159]. CD36 is responsible for the absorption and utilization of long-chain fatty acids, such as palmitic acid, which is the main primary source of energy for the heart [160]. Decreased expression of CD36 is associated with a shift towards glucose consumption in heart failure [161].

8.1.2. Metabolic adaptability in genotype-negative (G-) HCM

While one-third of symptomatic HCM cases are negative for sarcomeric mutations (G-/Ph+) [1,7], recent studies have identified genetic mutations in **mitochondrial** DNA and related **nuclear** DNA genes in these patients [162]. These mutations affect mitochondrial supercomplexes proteins, transfer RNA, and ribosomal RNA proteins [162]. However, it is still unclear whether these mutations are prevalent and relevant to HCM in general, as mitochondrial disorders often show symptoms comparable to other forms of cardiomyopathies.

It is unknown whether G- HCM patients, who are more likely to be obese and have LVOT blockage (HOCM) [9], experience the same declines in myocardial external efficiency and oxygen consumption as symptomatic G+ HCM patients [91]. The extra hemodynamic burden from septal bulging and mitral valve systolic anterior motion worsens cardiac function in HOCM [163,164] and has the potential to exacerbate the already high mitochondrial burden to maintain ATP regeneration, predisposing to the metabolic shift. Additionally, exercise may worsen the obstruction of the LVOT and further deteriorate cardiac function [1,165]. Nollet and colleagues [104] discovered recently a close relationship between mitochondrial dysfunction and septal hypertrophy in symptomatic G- HCM, which was associated with poor mitochondrial architecture. These findings suggest that genotype-negative HCM patients may have specific mitochondrial problems, but more research is needed to determine whether their cardiac efficiency and oxygen consumption are similar to those reported in symptomatic G+ HCM patients [91].

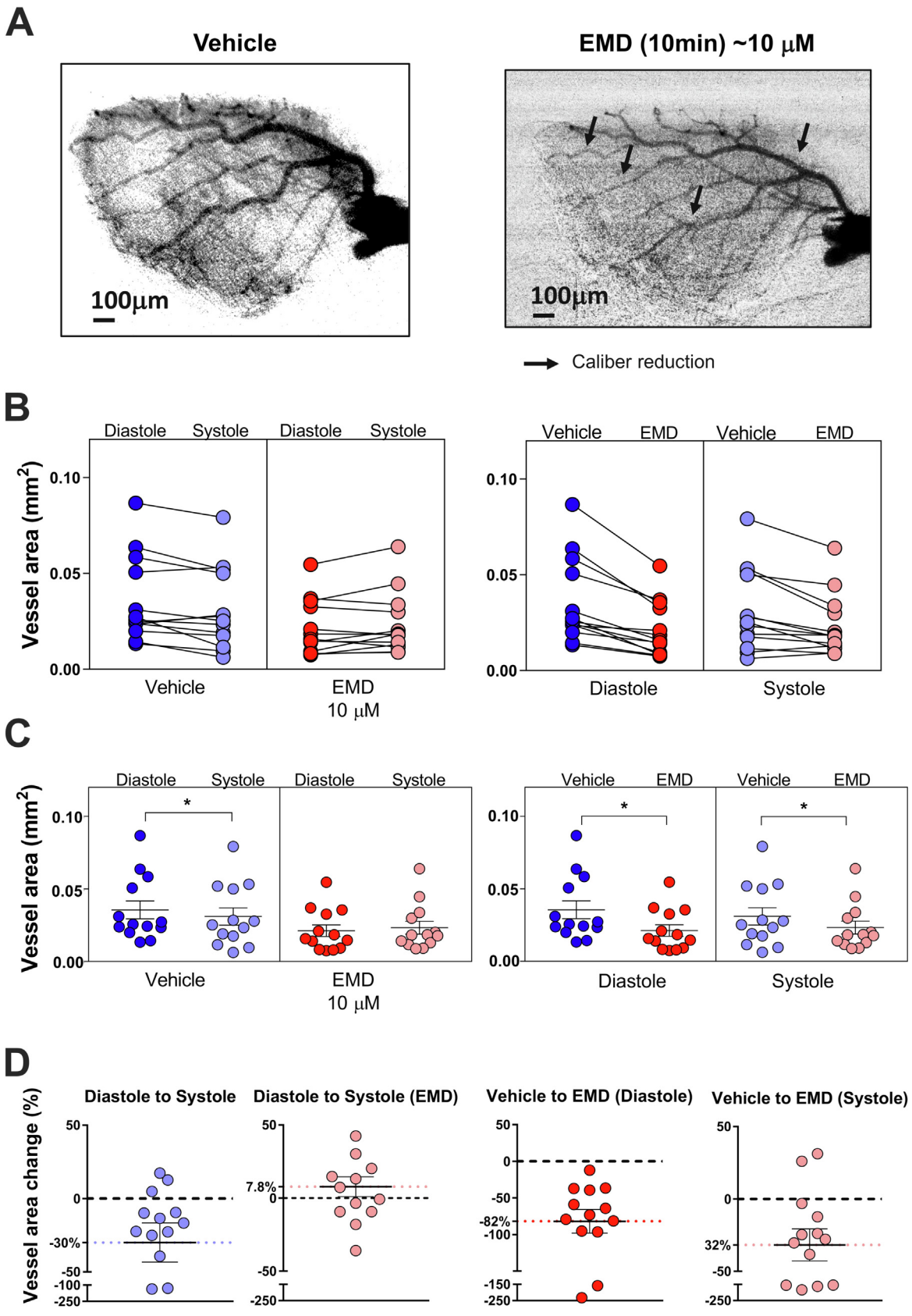
8.2. Changes to coronary perfusion and flow in HCM

In some situations, metabolic adaptation may occur associated with changes in myocardial oxygen availability, as microvascular dysfunction is frequently observed in individuals with manifested HCM [155–158]. **Microvascular dysfunction** has been identified as a predictor of death in human HCM [166]. In a healthy heart, coronary perfusion mostly occurs during diastole, while blood flow is reduced during systole due to constriction (compression) of coronary arteries embedded in the ventricular wall [167]. In HCM, however, the **hypercontractile** myocardium combined with slow ventricular relaxation, and excessive ventricular remodeling, are speculated to result in **inefficient** myocardial perfusion [155,156] and decreased coronary flow reserve [157,158]. Adverse survival rates have been reported in the context of LV hypertrophy and coronary artery disease [156,167], suggesting that increased cavity mass is a contributing factor that is indicative of myocardial ischemia in patients [168]. Ventricular remodeling involving intramyocardial vessel changes is a frequent finding in HCM [169,170], characterized by thickening of the tunica media with narrowing of the vessel lumen [169], and/or proliferation of intimal components of the vessel wall, i.e., vascular dysplasia [171]. This is most commonly observed in septal hypertrophy [169,172] and, more specifically, in HCM [171], and is frequently associated with microinfarctions during post-mortem histological examination [169].

The majority of observed alterations in microvascular function in HCM have been limited to symptomatic cases. Given the close relationship between coronary perfusion and the diastolic phase, it is often speculated that the observed microvascular dysfunction occurs early in HCM, and it is likely already present in asymptomatic cases directly affected by hypercontractility. This is suggested from the finding that impairment of microvascular function in HCM is more **closely linked** to the presence of sarcomeric mutations (G+) than to hypertrophy development in G- HCM [155,173], and sarcomeric HCM patients are frequently more hypercontractile than G- HCM patients [36,174]. We performed critical proof-of-concept experiments to investigate the potential role of hypercontractility on microvascular function, independent of cardiac remodeling. We found

Fig. 3. Mitochondrial protein expression of electron transport system components (OXPHOS), in mutant *Tnnt2*-I79N and *Mybpc3* hypertrophic cardiomyopathy (HCM) mouse hearts. Mice that carry the human c.772G > A *MYBPC3* mutation at the homozygous state (*Mybpc3*-KI) and WT controls were generated in a Black Swiss background as described previously (Vignier, Schlossarek et al. 2009) and used at an age of 12–16 weeks. Transgenic mice expressing mutant or the WT troponin T (*Tnnt2*-I79N and -WT) were generated as described previously (Miller, Szczesna et al. 2001) in a mixed genetic background and used at an age of 12–16 weeks. A. Western blot of the five mitochondrial complexes using the MitoProfile antibody cocktail (abcam, ab110413). The concentration of the antibody cocktail (1.5 mg/mL) was reduced 250× to obtain a final working concentration of 6.0 μg/mL. B—F. An unpaired *t*-Test was used to compare either I79N or *Mybpc3* to their respective wild-type controls. The significance level was set to $^*p < 0.05$. The data is presented as mean ± SEM and is normalized relative to controls (set to 100). $N = 4$ mouse hearts were used per each sample group and a rat heart homogenate (loading Ctrl) was used as loading control to normalize protein levels between blots. CI, complex I; CII, complex II; CIII, complex III; CIV, complex IV; CV, complex V.

a **direct link** between **hypercontractility** and **enhanced coronary flow constriction** using synchrotron microangiography imaging in mice that had been acutely perfused with the hypercontractility inducing drug EMD-57033 (Fig. 4). Under vehicle conditions, the net visualized coronary vessel area in wild-type mice dropped by 30 ± 13 % from diastole (0.036 ± 0.006 mm^2) to systole (0.031 ± 0.006 mm^2) (Figs. 4B-D),



consistent with physiological constriction of coronary arteries during the cardiac cycle. After EMD-57033 perfusion, diastolic coronary vessel area decreased by 82 ± 16 % from vehicle perfusion (0.036 ± 0.006 mm^2) to as low as 0.021 ± 0.004 mm^2, while systolic vessel area decreased by 32 ± 11 % from 0.031 ± 0.006 mm^2 to 0.023 ± 0.004 mm^2 (Figs. 4B-D). These findings are consistent with our hypothesis that hypercontractility induces pathological coronary artery constriction and demonstrate that sustained hypercontractility is likely sufficient to chronically decrease coronary flow, and potentially oxygen availability independent of other causes such as cardiac remodeling.

8.3. Exploring the complex relationship between catabolic and anabolic metabolism in HCM

A key metabolic endpoint in both heart failure and HCM is the metabolic shift from fatty acid to glucose oxidation. However, it is uncertain whether the persistent shift towards glucose is long-term beneficial to the HCM heart or simply a sign of disease progression. The complicated interplay of numerous processes, such as metabolite competition for energy production and cardiac growth, complicates our understanding of HCM pathogenesis even further. In particular, the competition between the metabolic pathways that increase energy demands for cardiac contraction (**catabolic metabolism**) and those that are essential for heart growth (**anabolic metabolism**). Both glucose and fatty acids provide energy to the heart, but they also have diverse effects on cardiac growth. Fatty acid oxidation, for example, inhibits cardiac growth stimulation, but glucose promotes protein synthesis during pathological cardiac growth [152]. In pressure-overload models, increased anabolic metabolism via glucose-derived uptake is required to sustain aspartate synthesis for hypertrophic growth [152].

While glucose uptake, rather than glucose oxidation, increases in symptomatic HCM patients [154], most glycolytic metabolism proteins are downregulated [126]. Notably, glycogen synthetase and mitochondrial hexokinase are upregulated in symptomatic HCM [126], implying that as HCM severity increases, glucose sustains anabolic pathways to account for increases in ventricular septum mass and hypertrophy development rather than being entirely utilized for energy generation. These findings are also consistent with early reports of increased glycogen deposits in symptomatic HCM [123–125], as well as AMPK downregulation [126], supporting that protein synthesis assists in hypertrophic development. Interestingly, large quantities of amino acids, particularly branched amino acids, but also ketones, have been found in the plasma of symptomatic HCM patients but not in individuals who are asymptomatic [175].

8.3.1. Exploring the potential role of ketones as alternative substrate sources

Recent investigations have explored alternative substrate sources for energy production in heart failure beyond glucose oxidation. Studies indicate that ketones, specifically β-hydroxybutyrate oxidation, could potentially become the primary source of ATP regeneration in the failing heart [176–178]. Although the role of ketones in manifested HCM is unknown, their energy yield is higher than that of fatty acids (but not glucose) [148], making them a potential alternative oxygen-efficient substrate for the energetically starved HCM heart.

**When referring to ketones, the phrase "bodies" is a historical artifact. Although the term "bodies" is often used in organic chemistry to describe insoluble particles, ketones are soluble in both blood and urine. Ketones are a result of acetyl-CoA metabolism in the liver and are released into the bloodstream to provide a substrate for ATP regeneration in extra-hepatic tissues during prolonged fasting or starvation.*

While plasma from symptomatic HCM patients contains significant quantities of ketones [175], proteomic data suggest that the usefulness of ketones as energy sinks in HCM may be limited due to the downregulation of multiple enzymes involved in ketone oxidation, including β-hydroxybutyrate dehydrogenase and 3-ketoacid CoA-transferase [126]. However, ketones may also play a diverse role in HCM, including signaling and gene expression. Research has shown that ketones have potential as signaling molecules, including their ability to act as HDAC1 inhibitors, which protect against oxidative stress [179], reduce hypertrophic signaling [180], and have anti-inflammatory effects by suppressing inflammasome activation [181]. The latter effects of ketones on HDAC inhibition are particularly intriguing, given the increase of HDAC4 in both symptomatic G+ and G-HCM [113].

While research on ketones in the failing heart, particularly in HCM, is still in its infancy, it provides a promising direction for investigating alternative sources beyond the conventional glycolytic-derived pathways involved in heart failure progression. This approach is critical in HCM, where previous studies have focused mainly on substrate metabolism in energy provision and contraction, while ignoring metabolic and signaling aspects that contribute to ventricular hypertrophy.

Promising therapies that target myofilament and mitochondrial function are emerging as potential treatments for HCM. Additionally, a newer class of metabolic-treatment drugs that affect overall cardiovascular and renal hemodynamics, inflammation, and myocardial Na^+ handling offer alternative strategies for treatment. Inhibitors of the Na^+/glucose co-transporter 2 (SGLT2), which are anti-obesity and anti-diabetic drugs, not only reduce cardiovascular and overall mortality rates in heart failure patients [182], but also increase blood ketone levels [183]. This increase in ketones may positively impact mitochondrial metabolism, oxidative stress and hypertrophy development in HCM.

9. Novel approaches for treating HCM

Treatment options for HCM mainly focus on managing symptoms, such as shortness of breath, angina, and arrhythmias, rather than addressing the **root cause**. Medications used to treat HCM include β-blockers, Ca^{2+} channel blockers, and ACE inhibitors, which can help reduce hypercontractility, lower blood pressure, and improve blood flow [163]. In cases with LVOT blockage, surgical procedures such as septal myectomy or alcohol septal ablation may be considered, and implantable cardioverter defibrillators can help manage the risk of sudden cardiac death [163]. Lifestyle changes such as weight loss may also be recommended, as excess body weight could contribute to HCM development [11]. However, these treatment options do not directly address the underlying pathophysiology of HCM. Perhexiline, a medication that was initially regarded as a metabolic therapeutic option for HCM, has been shown to improve exercise capacity

Fig. 4. Microvascular function using synchrotron microangiography. The coronary macro- and microvessels were evaluated in vivo by contrast microangiography in four eight-week-old wild-type C57BL6/J mice (internal caliber 30-100 μm). Synchrotron microangiography imaging was performed at the Spring-8 Radiation Research Facility in the Sayo District, Japan following protocols described previously (Pearson, Yoshimoto et al. 2017, Katare, Pearson et al. 2018). The acute intravenous administration of the Calcium-sensitizer EMD-57033 allowed us to investigate the role of hypercontractility on coronary microvessel function. EMD-57033 (1.6 mg/kg) was dissolved in 1 % DMSO, 30 % PEG, 1 % Tween80 and 68 % saline (*v*/v%). A. Left, To produce baseline cine-angiograms a vehicle solution was infused through the jugular vein for 10 min. A. Right, EMD-57033 was then infused for 10 min and imaging repeated. For image filtering, background removal, and vessel area measurements, Fiji/ImageJ 2.9.0/1.53 t was utilized. B. In the absence and presence of EMD-57033, three to four of the microvessels per mouse were quantified as visualized vessel area during diastole and systole. C. and D. Vehicle conditions, visualized coronary vascular area decreased from 0.036 ± 0.006 mm^2 in diastole to 0.031 ± 0.006 mm^2 in systole (i.e., by 30 ± 13 %), consistent with physiological constriction of coronary arteries. After EMD-57033, diastolic coronary vessel area decreased by 82 ± 16 % from vehicle perfusion (0.036 ± 0.006 mm^2) to as low as 0.021 ± 0.004 mm^2, while systolic vessel area decreased by 32 ± 11 % from 0.031 ± 0.006 mm^2 to 0.023 ± 0.004 mm^2. These findings are consistent with potentiated coronary artery constriction caused by hypercontractility. The vascular area did not change from diastole (0.021 ± 0.004 mm^2) to systole (0.023 ± 0.004 mm^2) during EMD-57033 perfusion. $N = 4$ animals were used for microangiography imaging, and the number of microvessels analyzed per mouse ranged from three to four (total of $n = 13$ microvessels). The data is presented as mean ± SEM and a paired *t*-test was conducted. The significance level was set to $^*p < 0.05$.

[184] and reduce ventricular hypertrophy in HCM animals [185]. Perhexiline's capacity to shift substrate usage from fatty acids to glucose was initially postulated to play a role in the drug's effects [184]. However, decreased oxygen availability and lower reliance on mitochondrial oxygen via glucose-driven oxidation to maximize ATP regeneration is likely a consequence of HCM progression. Instead, the effects of perhexiline are likely pleiotropic, and may be attributable to its ability to enhance the activity of anti-oxidant enzymes and decrease oxidative stress [185–187], which may aid in rebalancing the redox state and reducing ventricular mass in HCM.

It is essential to consider selective treatment strategies that address mechano-energetic uncoupling, mitochondrial dysfunction and the rise in oxidative stress to prevent or reverse the progression of HCM, hypertrophy, and arrhythmia susceptibility. In addition, since weight loss improves myocardial energetics [188], metabolic drugs are likely favorable for the treatment of patients with HCM. These treatment strategies are discussed next.

9.1. NAC for the treatment of HCM

In recent years, there has been growing interest in the potential of anti-oxidant and mitochondria-targeted therapies for preventing or reversing HCM progression. One such therapy that has been investigated is *N*-acetylcysteine (NAC), an **anti-oxidant** that has been shown to protect the heart from HCM-related injury in mice [38,109,189,190] (Fig. 1). NAC serves as a precursor of **glutathione**, which is the largest intracellular thiol pool that protects against oxidative stress [191,192]. Specifically, NAC has been found to lower lipid peroxidation, myocardial fibrosis, cellular disarray, and hypertrophy development, while also improving diastolic function [38,109,189,190]. NAC treatment was also shown to restore the cardiac phenotype in HCM models while also normalizing the level of oxidized glutathione [79]. Furthermore, NAC treatment has been observed to inhibit the MAPK signaling system, which is a known principal contributor to the development of concentric hypertrophy in HCM [115]. In HCM animals, NAC treatment decreased myofilament oxidation (lowered s-glutathionylation of cMyBP-C) and hypercontractility [38]. These promising findings suggest that NAC may have the potential as a therapeutic option for HCM.

9.2. Mitochondria-targeted treatments in HCM

9.2.1. Benefits of SS-31

Mitochondrial dysfunction is a key factor in the development of HCM in humans [103,104] and treatments targeting mitochondrial function have been investigated. The integrity of the mitochondrial respiratory chain is challenged when the mitochondrial membrane is degraded by lipid peroxidation, resulting in decreased ATP generation and increased ROS formation. **Cardiolipin**, a phospholipid found in both the inner (up to 22 %) and outer (3 %) mitochondrial membranes, is an essential component of the mitochondrial membrane [193–195]. Cardiolipin supports mitochondrial supercomplexes and electron transfer flow for ATP regeneration, while decreasing electron leakage and ROS formation (Fig. 5) [196]. Cardiolipin, on the other hand, is particularly sensitive to lipid peroxidation and formation of 4-HNE, which disrupts mitochondrial supercomplexes and impairs ATP generation while increasing ROS production [197]. The cohesiveness of mitochondrial supercomplexes is disrupted when cardiolipin is damaged, and essential molecules such as cytochrome *c* (Cytc) and coenzyme Q (CoQ or Q) are depleted (Fig. 5, middle panel) [198]. The Szeto-Schiller peptide (**SS-31** or **elamipretide**) is a **mitochondria-targeted peptide** that binds to cardiolipin and accumulates in the mitochondria, improving the curvature of the inner mitochondrial membrane and bringing the supercomplexes closer together [199]. This reduces electron leakage during electron transfer reactions, decreasing excessive ROS production and enhancing ATP regeneration (Fig. 5, lower panel) [200,201]. Animal studies have demonstrated that SS-31 treatment reduces mortality in pressure-overload models [107], as well as HCM animal models, while **preventing** arrhythmia episodes [144]. Recent ex vivo experiments on human HCM biopsies have shown that SS-31 treatment improves the cohesiveness of mitochondrial respirasome complexes and increases complex I stability [104]. These findings suggest that improving mitochondrial function with SS-31 could be a promising treatment strategy for HCM.

9.2.2. Benefits of CoQ

Coenzyme Q (CoQ) is an essential lipid-soluble molecule for the **transport of electrons** between complexes I and III in the mitochondria, which are necessary for energy production (Fig. 5). Cardiolipin instability in the inner mitochondrial membrane can lead to depletion of CoQ. **CoQ therapy** reduces symptoms of HCM, such as fatigue and dyspnea, while also decreasing septal thickness [202]. Additionally, administration of CoQ to HCM patients significantly improved their NYHA class, quality of life, and diastolic function [203]. CoQ therapy in patients with HOCM also reduced the gradient of the LV outflow tract and septum mass [203]. The benefits of CoQ therapy in HCM are likely due to its ability to restore the energetic-redox equilibrium by scavenging electrons from the inner mitochondrial membrane, and thereby preventing excessive ROS production.

9.2.3. Benefits of NAD^+ supplementation

Numerous studies suggest that **NAD^+ boosters** have promising potential as a treatment option for heart failure. Restoring cellular NAD^+ levels in mouse models of aging, metabolic syndrome, and hypertension was found to improve both diastolic function and mitochondrial bioenergetics [204]. Additionally, nicotinamide riboside therapy, a **precursor** to NAD^+, was shown to prevent the onset of heart failure in dilated cardiomyopathy mice [205]. In HCM biopsies [126], changes in NAD^+ homeostasis have been observed, implying reduced NADH availability. The addition of NAD^+ to fresh HCM biopsies enhances mitochondrial respiration by potentially increasing mitochondrial NADH availability (Figs. 1 and 5, lower panel) [104]. These data support the potential use of NAD^+ supplementation in various HCM models, and eventually in individuals with HCM.

9.3. Hypercontractility in HCM: the potential benefits of myosin deactivators as treatment

A new class of compounds has been developed to target the disease-causing mechanism of HCM by inhibiting the myosin ATPase and reducing hypercontractility of the myofilaments [206]. The first of its class, **Mavacamten**, is a myosin inactivator that improves diastolic function by stabilizing the super relaxed state (SRX) of the myosin molecule, which is an energy-conserving state, inducing a negative inotropic effect and reducing the energetic load on the HCM myocardium [207].

during myofilament contraction, myosin transits between three functioning biochemical states. The **(1) actin-bound state, which has a rapid ATP turnover, and two unbound states with extremely low ATP turnover: **(2)** the disorder relaxed state (DRX), which has approximately 100-fold less activity than the actin-bound state, and **(3)** the super relaxed state (SRX), which has an additional 10-fold decrease in activity [207]. Up to 60 % of unbound myosin heads are held "in reserve" in the energy-conserving SRX state.*

Mavacamten was shown to reverse hypercontractility, maladaptive cardiac remodeling, myofibrillar disarray, and fibrosis in animal models of HCM [206] as well as in patients with HCM [208,209]. In clinical trials, Mavacamten reduced the LVOT obstruction in patients with HOCM, improving symptoms, exercise capacity and LV geometry [208–211]. Further compounds with a similar mode of action are currently under development and some have entered clinical trials [212]. Mavacamten and related drugs offer prospective HCM therapy alternatives by addressing the underlying cause of the disease as early as possible.

9.4. Gene editing as a potential therapy for HCM

CRISPR **gene editing** is a promising technology for treating hereditary cardiomyopathies, including HCM. The success of CRISPR editing in

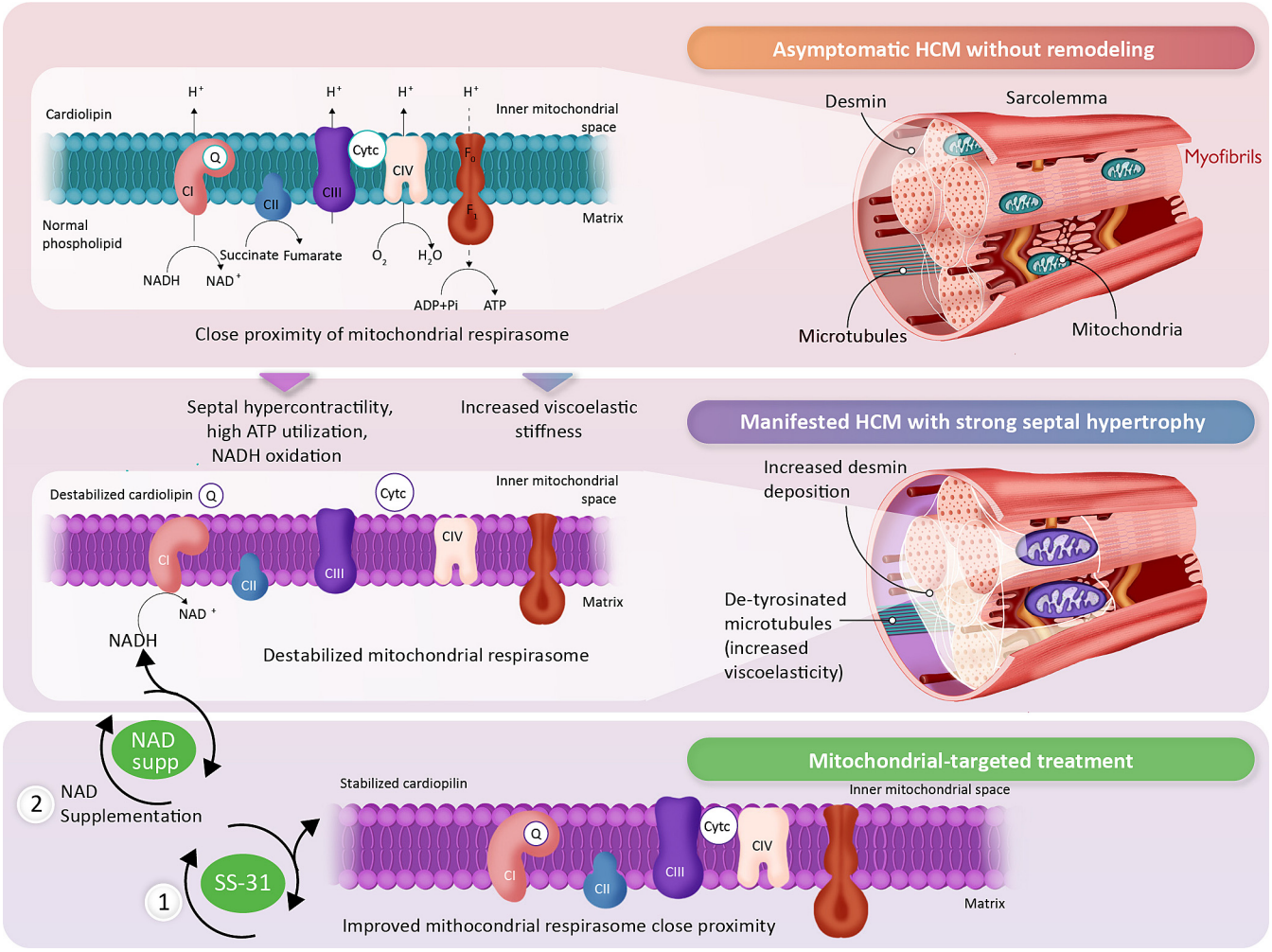


Fig. 5. Mitochondria-targeted therapies in hypertrophic cardiomyopathy (HCM). The top panel illustrates a healthy cardiomyocyte-mitochondrial organization in asymptomatic HCM individuals. The cardiomyocyte-mitochondrial organization in HCM is characterized by near normal production of viscoelastic proteins, such as desmin and microtubules, and the close proximity of mitochondrial supercomplexes or respirasomes in the presence of cardiolipin. The inner mitochondrial membrane (IMM) is traversed by NADH dehydrogenase (complex I, CI), cytochrome *b*–c1 complex (complex III, CIII), cytochrome *c* oxidase (complex IV, CIV), and F_oF_1-ATP synthase (complex V). Reduced forms of NADH and $FADH_2$ (derived from succinate) donate electrons to the electron transport system via CI or CII (not membrane spanning), which are sequentially transferred to electron carriers, such as coenzyme Q (CoQ or Q), CIII, and cytochrome *c* (Cytc). CIV accepts electrons from the electron transport system and transforms oxygen into water. As electrons move along the electron transfer system, protons (H^+) are pumped from the mitochondrial matrix into the mitochondrial IMM (at CI, CIII, and CIV; CII lacks a proton pumping mechanism), establishing an electrochemical proton gradient across the IMM. This gradient is used to regenerate ATP from ADP at complex V. The **middle panel** illustrates the potential consequences of septal hypercontractility, high ATP utilization and increased NADH oxidation, to induce septal enlargement in manifested HCM individuals. This progression is characterized by increased production of viscoelastic elements, such as microtubule densification, desmin deposition, and hypertrophied myofibrils. Consequently, this results in poor myofibril and mitochondrial architecture, and disorganized mitochondrial respirasomes. The **lower panel** highlights the potential benefits of improving the provision of reducing equivalents in the Krebs cycle with NAD^+ supplementation or improving respiratory chain function with elamipretide to stabilize cardiolipin in the IMM. These therapies may improve the proximity of mitochondrial respirasomes, reduce electron leakage and oxidative stress, and enhance ATP regeneration. Image adapted with permission from Sequeira et al. Eur Heart J. 2023 (Sequeira, Batzner et al. 2023).

repairing genetic defects in other diseases such as Duchenne Muscular Dystrophy (DMD) [213] has led to exploration of its potential in treating HCM. DMD is a severe genetic disease caused by mutations in the dystrophin gene, leading to early death from cardiac and respiratory failure. CRISPR gene editing has been shown to correct DMD mutations and restore dystrophin production in skeletal and cardiac muscle [213]. In two recent pre-clinical studies [214,215], researchers gene-edited the human HCM missense mutation in the *MYH7* gene that encodes the MyHC-R403Q mutation. Both studies used CRISPR-Cas9 approaches to achieve highly efficient and precise correction of the pathogenic variant in human-derived HCM cells, with reduction of hypercontractility and improved mitochondrial oxygen consumption, while in HCM models CRISPR gene-editing of the mutation decreased cardiac hypertrophy and fibrosis [214,215]. Challenges remain in optimizing mutation-specific gene editing, improving gene editing precision, delivering editing components throughout the muscle, and minimizing immunological reactions. The prospect of using single-dose definitive therapies for HCM through CRISPR or other gene editing alternatives presents an encouraging avenue for addressing another root cause of HCM.

9.5. Novel treatment strategies for HCM

Alternative therapeutic approaches for HCM should be examined, such as metabolic focused therapies that have been demonstrated to improve patient outcomes in other heart failure disorders. Weight loss, for example, improves myocardial energetics in obese patients [188], implying that metabolic treatment approaches may be effective in treating myocardial energy depletion in HCM. The use of sodium glucose cotransporter 2 (**SGLT2**)

inhibitors, which have shown encouraging outcomes in heart failure studies, is one such targeted-treatment approach, although their efficacy in HCM is currently unknown and hence merits investigation.

9.5.1. SGLT2-inhibitors

SGLT2 inhibitors have revolutionized metabolic management for heart failure patients, demonstrating improved outcomes across all heart failure types [182]. The SGLT2 inhibitor empagliflozin induces a starvation-like metabolism by removing glucose via the kidney [216], which reduces heart failure hospitalization rates and lowers cardiovascular mortality regardless of comorbidities [217]. SGLT2 inhibitors appear to influence cardiovascular and renal hemodynamics, inflammation [218], and cardiomyocyte Na^+ handling directly [219] or indirectly through CaMKII inhibition [220,221]. Also, they enhance cardiac, renal, and systemic metabolism. Improving Na^+ handling may help mitigate the arrhythmogenic effects of CaMKII hyperactivation and the ROS-induced increase in the late Na^+ current ($I_{Na,L}$) observed in HCM (see Section 7.2.2) [136].

Some SGLT2 inhibitors have been shown to inhibit the sarcolemmal Na^+/H^+ exchanger (NHE-1), leading to a reduction in cytosolic Na^+ concentrations in cardiomyocytes and increasing mitochondrial Ca^{2+} levels [222,223]. The increase in mitochondrial Ca^{2+} may prevent the rise in oxidative stress and arrhythmias in HCM, since an increase in mitochondrial Ca^{2+} is required to stimulate Krebs cycle dehydrogenases for the regeneration of NADH under increased workload or stress, this may facilitate the matching of the increase in ATP demand to an increase in ATP production at the respiratory chain (see Section 6) [106]. In addition, SGLT2 inhibitors are known to raise blood ketone levels [183], suggesting that they could be a useful treatment for disrupted mitochondrial metabolism, oxidative stress, and hypertrophy development in HCM. The ongoing EMPA-VISION trial is currently evaluating whether empagliflozin can improve myocardial PCr/ATP ratio [224]. If successful, this could be a promising approach to improve the observed reduction in PCr/ATP ratio seen in animal models [87,88,92] and humans [90] with HCM.

10. Beyond sarcomeric mutations: mechano-energetic uncoupling and metabolic factors contributing to HCM pathophysiology

The relevance of mechano-energetic uncoupling and metabolic dysregulation cannot be overlooked in the context of HCM, as various forms of the disease are associated with such dysregulations, in addition to HCM with sarcomeric mutations (G+). Other causes of HCM include variations in genes encoding proteins that are involved in metabolism, mitochondrial function, and pro-hypertrophic signaling [1]. As discussed earlier in Section 7.1.3, insights from other types of cardiomyopathies, such as PRKAG2 and GSDIIIa syndromes, can inform about the pathophysiological mechanisms contributing to the transition from asymptomatic to symptomatic HCM with sarcomeric mutations (G+). This cross-talk of knowledge might prove invaluable in the development of effective treatment strategies for HCM.

Barth syndrome cardiomyopathy is an example of a mitochondrial cardiomyopathy characterized by diastolic dysfunction and hypertrophy, and is sometimes accompanied by LV non-compaction [225]. Mutations in the gene encoding tafazzin, a mitochondrial transacylase involved in cardiolipin biogenesis, result in cardiolipin synthesis defects. Cardiolipin remodeling in Barth syndrome destabilizes the mitochondrial Ca^{2+} uniporter complex, preventing mitochondrial Ca^{2+} uptake and activation of the Krebs cycle [226]. Consequently, this causes mechano-energetic uncoupling, hypercontractility, and arrhythmias [226]. Gene-editing therapy with human *TAZ* was demonstrated to reverse the cardiomyopathy phenotype in mice [227]. Despite failing to improve the trial's primary endpoint (6-min walk test), **elamipretide** improved stroke volume and well-being in patients with Barth syndrome [228]. Ongoing clinical investigations are focusing on interventions that target fatty acid metabolism [229]. Although Barth syndrome is a rare disease, the mechanistic insights from selective cardiolipin defects may also be applicable to HCM with sarcomeric mutations, early phases of diabetes [230,231], and ischemia/reperfusion injury [232], where cardiolipin remodeling is substantial [233].

The **Noonan syndrome** is another cause of HCM. It is a multisystemic disorder caused by mutations in genes linked to the Ras-Raf-MEK1/2 pathway, i.e., RASopathies, which converge on ERK1/2 signaling [234,235]. In mouse models of Noonan syndrome, interfering with ERK1/2 [236] or MEK1/2 [237,238] improves cardiac function. MEK inhibition with **trametinib**, typically used to treat specific types of cancer with RAS/MAPK pathway activation, ameliorated LV hypertrophy in Noonan syndrome patients [239]. To selectively target hypertrophy without cardiotoxic side effects, the development of specific ERK1/2-inhibitors that interfere with ERK-dimerization at the nucleo-cytosolic interface may be advantageous as a potential therapeutic concept for various forms of HCM [240].

Fabry disease is a rare X-linked inherited lysosomal storage disorder characterized by deficient α-galactosidase A activity, which results in the accumulation of globotriasylceramide (Gb3) in affected tissues such as the heart [241]. This causes LV hypertrophy, myocardial fibrosis, diastolic dysfunction, and arrhythmias. Enzyme replacement therapy prevents cardiac complications and delay disease progression [241]. Similar to HCM with sarcomeric mutations, myocardial energy deprivation with decreased PCr/ATP ratio is typically observed and closely correlates with the extent of LV hypertrophy and fibrosis [242,243]. This metabolic defect is reversible after sphingolipid clearance by enzyme replacement therapy with the administration of α-Gal A [243].

Although hereditary forms of HCM have distinct etiologies, which in some cases can be specifically targeted with tailored treatments, they may share common pathophysiological mechanism paths that drive disease progression through a vicious cycle of mechano-energetic uncoupling, oxidative stress and metabolic dysregulation.

11. Conclusion

The pathogenesis of HCM is multifactorial and involves various cellular perturbations that contribute to diastolic dysfunction and remodeling of the HCM heart. Sarcomeric mutations or secondary mutation-induced cellular perturbations play a crucial role in the impaired relaxation of the myocardium. Hypercontractility lies upstream of several key derangements including increased mitochondrial workload, and oxidative stress, contributing to energy depletion and mechano-energetic uncoupling, driving growth of myocardial mass through signaling pathways such as ERK and, potentially, suppression of AMPK. In HCM, metabolic remodeling also occurs, characterized by decreased fatty acid oxidation, increased glucose uptake and decreased oxygen utilization in the myocardium. Moreover, under certain conditions, alternative metabolic sources such as ketones may offer an energy supply for the heart, while also serving as potential signaling molecules that can mitigate oxidative stress and regulate hypertrophic signaling. In addition, arrhythmias are frequently triggered in HCM by the high Ca^{2+}-buffering at the myofilaments and changes in the ATP/ADP ratio. Understanding the mechanisms that drive the progression of HCM is crucial in the development of effective therapeutic strategies. Targeting the underlying mechano-energetic and metabolic derangements may provide potential treatment alternatives to improve HCM course beyond what is now available.

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CRediT authorship contribution statement

All authors approved the final version of the manuscript. Conception and design of the experiments: V.S. devised, designed and performed the project, analyzed data and wrote the manuscript. M.W. performed experiments, analyzed data and revised the manuscript; H.T. performed

experiments, analyzed data and revised the manuscript; C.M. supervised and funded the project, wrote and revised the manuscript; J.T.P. performed experiments, analyzed data, revised the manuscript and funded the project.

Ethics statement

All experiments involving animals were conducted in accordance with guidelines of Physiological Society of Japan and approved by the animal experimentation committee at the National Cerebral and Cardiovascular Center (Proposal Nos. 220,042). The reporting of animal studies in this article complies with the ARRIVE guidelines for animal research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, Hagege AA, Lafont A, Limongelli G, Mahrholdt H, McKenna WJ, Mogensen J, Nihoyannopoulos P, Nistri S, Pieper PG, Pieske B, Rapezzi C, Rutten FH, Tillmanns C, Watkins H. ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy. Eur Heart J. 2014;35(2014):2733–79.

[2] Akhtar M, Elliott P. The genetics of hypertrophic cardiomyopathy. Glob Cardiol Sci Pract. 2018;2018(3):36.

[3] Watkins H. Sudden death in hypertrophic cardiomyopathy. N Engl J Med. 2000;342:422–4.

[4] Tardiff JC, Carrier L, Bers DM, Poggesi C, Ferrantini C, Coppini R, Maier LS, Ashrafian H, Huke S, van der Velden J. Targets for therapy in sarcomeric cardiomyopathies. Cardiovas Res. 2015;105:457–70.

[5] Ashrafian H, Redwood C, Blair E, Watkins H. Hypertrophic cardiomyopathy:a paradigm for myocardial energy depletion. Trends Genet. 2003;19(5):263–8.

[6] Maron BJ, Olivotto I, Spirito P, Casey SA, Bellone P, Gohman TE, Graham KJ, Burton DA, Cecchi F. Epidemiology of hypertrophic cardiomyopathy-related death: revisited in a large non-referral-based patient population. Circulation. 2000;102:858–64.

[7] Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, Benaiche A, Isnard R, Dubourg O, Burban M, Gueffet J-P, Millaire A, Desnos M, Schwartz K, Hainque B, Komajda M. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. Circulation. 2003;107:2227–32.

[8] Michels M, Olivotto I, Asselbergs FW, van der Velden J. Life-long tailoring of management for patients with hypertrophic cardiomyopathy: awareness and decision-making in changing scenarios. Neth Heart J. 2017;25(3):186–99.

[9] de Feria AE, Kott AE, Becker JR. Sarcomere mutation negative hypertrophic cardiomyopathy is associated with ageing and obesity. Open Heart. 2021.;8(1).

[10] Biagini E, Coccolo F, Ferlito M, Perugini E, Rocchi G, Bacchi-Reggiani L, Lofiego C, Boriani G, Prandstraller D, Picchio FM, Branzi A, Rapezzi C. Dilated-hypokinetic evolution of hypertrophic cardiomyopathy: prevalence, incidence, risk factors, and prognostic implications in pediatric and adult patients. J Am Coll Cardiol. 2005;46:1543–50.

[11] Robertson J, Schaufelberger M, Lindgren M, Adiels M, Schiöler L, Torén K, McMurray J, Sattar N, Åberg M, Rosengren A. Higher body mass index in adolescence predicts cardiomyopathy risk in midlife. Circulation. 2019;140(2):117–25.

[12] Kubo T, Kitaoka H, Okawa M, Hirota T, Hayato K, Yamasaki N, Matsumura Y, Yabe T, Doi YL. Gender-specific differences in the clinical features of hypertrophic cardiomyopathy in a community-based japanese population: results from Kochi RYOMA study. J Cardiol. 2010;56(3):314–9.

[13] Lin C-L, Chiang C-W, Shaw C-K, Chu P-H, Chang C-J, Ko Y-L. Gender differences in the presentation of adult obstructive hypertrophic cardiomyopathy with resting gradient a study of 122 patients. Jpn Circ J. 1999;63(11):859–64.

[14] Olivotto I, Maron MS, Adabag AS, Casey SA, Vargiu D, Link MS, Udelson JE, Cecchi F, Maron BJ. Gender-related differences in the clinical presentation and outcome of hypertrophic cardiomyopathy. J Am Coll Cardiol. 2005;46:480–7.

[15] Page SP, Kounas S, Syrris P, Christiansen M, Frank-Hansen R, Andersen PS, Elliott PM, McKenna WJ. Cardiac myosin binding protein-C mutations in families with hypertrophic cardiomyopathy. Circ Cardiovasc Genet. 2012;5(2):156–66.

[16] Najafi A, Schlossarek S, van Deel ED, van den Heuvel N, Güçlü A, Goebel M, Kuster DWD, Carrier L, van der Velden J. Sexual dimorphic response to exercise in hypertrophic cardiomyopathy-associated MYBPC3-targeted knock-in mice. Pflügers Archiv. 2014;467(6):1303–17.

[17] Schober T, Huke S, Venkataraman R, Gryshchenko O, Kryshtal D, Hwang HS, Baudenbacher FJ, Knollmann BC. Myofilament ca sensitization increases cytosolic ca binding affinity, alters intracellular ca homeostasis, and causes pause-dependent ca-triggered arrhythmia. Circ Res. 2012;111:170–9.

[18] Knollmann BC, Kirchhof P, Sirenko SG, Degen H, Greene AE, Schober T, Mackow JC, Fabritz L, Potter JD, Morad M. Familial hypertrophic cardiomyopathy-linked mutant troponin T causes stress-induced ventricular tachycardia and Ca2+-dependent action potential remodeling. Circ Res. 2003;92:428–36.

[19] Najafi A, Schlossarek S, van Deel ED, van den Heuvel N, Güçlü A, Goebel M, Kuster DWD, Carrier L, van der Velden J. Sexual dimorphic response to exercise in hypertrophic cardiomyopathy-associated MYBPC3-targeted knock-in mice. Pflugers Arch. 2014:1–15.

[20] Evans CC, Pena JR, Phillips RM, Muthuchamy M, Wieczorek DF, Solaro RJ, Wolska BM. Altered hemodynamics in transgenic mice harboring mutant tropomyosin linked to hypertrophic cardiomyopathy. Am J Physiol. 2000;279:H2414–23.

[21] Chandra M, Rundell VLM, Tardiff JC, Leinwand LA, de Tombe PP, Solaro RJ. Ca2+ activation of myofilaments from transgenic mouse hearts expressing R92Q mutant cardiac troponin T. Am J Physiol. 2001;280:H705–13.

[22] Nagueh SF, Kopelen HA, Lim D-S, Zoghbi WA, Quiñones MA, Roberts R, Marian AJ. Tissue doppler imaging consistently detects myocardial contraction and relaxation abnormalities, irrespective of cardiac hypertrophy, in a transgenic rabbit model of human hypertrophic cardiomyopathy. Circulation. 2000;102:1346–50.

[23] Tardiff JC, Hewett TE, Palmer BM, Olsson C, Factor SM, Moore RL, Robbins J, Leinwand LA. Cardiac troponin T mutations result in allele-specific phenotypes in a mouse model for hypertrophic cardiomyopathy. J Clin Inv. 1999;104:469–81.

[24] Fraysse B, Weinberger F, Bardswell SC, Cuello F, Vignier N, Geertz B, Starbatty J, Krämer E, Coirault C, Eschenhagen T, Kentish JC, Avkiran M, Carrier L. Increased myofilament Ca2+ sensitivity and diastolic dysfunction as early consequences of MYBPC3 mutation in heterozygous knock-in mice. J Mol Cell Cardiol. 2012;52:1299–307.

[25] Knollmann BC, Blatt SA, Horton K, de Freitas F, Miller T, Bell M, Housmans PR, Weissman NJ, Morad M, Potter JD. Inotropic stimulation induces cardiac dysfunction in transgenic mice expressing a troponin T (I79N) mutation linked to familial hypertrophic cardiomyopathy. J Biol Chem. 2001;276:10039–48.

[26] Kim S-J, Iizuka K, Kelly RA, Geng Y-J, Bishop SP, Yang G, Kudej A, McConnell BK, Seidman CE, Seidman JG, Vatner SF. An α-cardiac myosin heavy chain gene mutation impairs contraction and relaxation function of cardiac myocytes. Am J Physiol. 1999;276:H1780–7.

[27] Ho CY, Carlsen C, Thune JJ, Havndrup O, Bundgaard H, Farrohi F, Rivero J, Cirino AL, Andersen PS, Christiansen M, Maron BJ, Orav EJ, Køber L. Echocardiographic strain imaging to assess early and late consequences of sarcomere mutations in hypertrophic cardiomyopathy. Circ Cardiovas Gen. 2009;2:314–21.

[28] Germans T, Rüssel I, Götte M, Spreeuwenberg M, Doevendans P, Pinto Y, van der Geest R, van der Velden J, Wilde A, van Rossum A. How do hypertrophic cardiomyopathy mutations affect myocardial function in carriers with normal wall thickness?Assessment with cardiovascular magnetic resonance. J Cardiovas Mag Res. 2010.;12(13).

[29] Nagueh SF, Bachinski LL, Meyer D, Hill R, Zoghbi WA, Tam JW, Quiñones MA, Roberts R, Marian AJ. Tissue doppler imaging consistently detects myocardial abnormalities in patients with hypertrophic cardiomyopathy and provides a novel means for an early diagnosis before and independently of hypertrophy. Circulation. 2001;104:128–30.

[30] Wigle ED, Rakowski H, Kimball BP, Williams WG. Hypertrophic cardiomyopathy. Clinical spectrum and treatment. Circulation. 1995;92:1680–92.

[31] Betocchi S, Hess OM, Losi MA, Nonogi H, Krayenbuehl HP. Regional left ventricular mechanics in hypertrophic cardiomyopathy. Circulation. 1993;88:2206–14.

[32] Hayashida W, Kumada T, Kohno F, Noda M, Ishikawa N, Kojima J, Himura Y, Kawai C. Left ventricular regional relaxation and its nonuniformity in hypertrophic nonobstructive cardiomyopathy. Circulation. 1991;84:1496–504.

[33] Maras D, Chung R, Duncan A, Li W, Thorp C, Stellan M, Lindqvist P, Henein MY. Patterns of cardiac dysfunction coinciding with exertional breathlessness in hypertrophic cardiomyopathy. Int J Cardiol. 2013;170:233–8.

[34] Wijnker P, Sequeira V, Kuster DW, van der Velden J. Hypertrophic cardiomyopathy: a vicious cycle triggered by sarcomere mutations and secondary disease hits. Antioxid Redox Signal. 2018;31:318–58.

[35] Sequeira V, Najafi A, Wijnker P, Michels M, Remedios CD, Kuster DWD, van der Velden J. ADP-stimulated contraction: a predictor of thin-filament activation in cardiac disease. Proc Natl Acad Sci. 2015;112:E7003–12.

[36] Sequeira V, Wijnker PJM, Nijenkamp LLAM, Kuster DWD, Najafi A, Witjas-Paalberends ER, Regan JA, Boontje N, ten Cate FJ, Germans T, Carrier L, Sadayappan S, van Slegtenhorst MA, Zaremba R, Foster DB, Murphy AM, Poggesi C, dos Remedios C, Stienen GJM, Ho CY, Michels M, van der Velden J. Perturbed length-dependent activation in human hypertrophic cardiomyopathy with missense sarcomeric gene mutations. Circ Res. 2013;112:1491–505.

[37] Flenner F, Friedrich FW, Ungeheuer N, Christ T, Geertz B, Reischmann S, Wagner S, Stathopoulou K, Sohren KD, Weinberger F, Schwedhelm E, Cuello F, Maier LS, Eschenhagen T, Carrier L. Ranolazine antagonizes catecholamine-induced dysfunction in isolated cardiomyocytes, but lacks long-term therapeutic effects in vivo in a mouse model of hypertrophic cardiomyopathy. Cardiovasc Res. 2016;109(1):90–102.

[38] Wilder T, Ryba DM, Wieczorek DF, Wolska BM, Solaro RJ. N-acetylcysteine reverses diastolic dysfunction and hypertrophy in familial hypertrophic cardiomyopathy. Am J Physiol. 2015;309:H1720–30.

[39] Ferrara C, Witjas-Paalberends ER, Piroddi N, Scellini B, Tesi C, Sequiera V, dos Remedios C, Schlossarek S, Leung J, Carrier L, Redwood C, Marston S, van der Velden J, Poggesi C. The HCM-associated cardiac troponin T mutation K280N increases the energetic cost of tension generation in human cardiac myofibrils. Biophys J. 2013;104:187a.

[40] Sequeira V, Wijnker PJ, Nijenkamp LL, Kuster DW, Najafi A, Witjas-Paalberends ER, Regan JA, Boontje N, Ten Cate FJ, Germans T, Carrier L, Sadayappan S, van

Slegtenhorst MA, Zaremba R, Foster DB, Murphy AM, Poggesi C, Dos Remedios C, Stienen GJ, Ho CY, Michels M, van der Velden J. Perturbed length-dependent activation in human hypertrophic cardiomyopathy with missense sarcomeric gene mutations. Circ Res. 2013;112(11):1491–505.
[41] Schuldt M, Johnston JR, He H, Huurman R, Pei J, Harakalova M, Poggesi C, Michels M, Kuster DWD, Pinto JR, van der Velden J. Mutation location of HCM-causing troponin T mutations defines the degree of myofilament dysfunction in human cardiomyocytes. J Mol Cell Cardiol. 2021;150:77–90.
[42] Sequeira V, Wang L, Wijnker PJM, Kim K, Pinto JR, dos Remedios C, Redwood C, Knollmann BC, van der Velden J. Low expression of the K280N TNNT2 mutation is sufficient to increase basal myofilament activation in human hypertrophy cardiomyopathy. J Mol Cell Cardiol Plus. 2022;1:100007.
[43] Watkins H, Rosenzweig A, Hwang D-S, Levi T, McKenna W, Seidman CE, Seidman JG. Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. N Engl J Med. 1992;326:1108–14.
[44] Watkins H, McKenna WJ, Thierfelder L, Suk HJ, Anan R, O'Donoghue A, Spirito P, Matsumori A, Moravec CS, Seidman JG, Seidman CE. Mutations in the genes for cardiac troponin T and α-tropomyosin in hypertrophic cardiomyopathy. New Eng J Med. 1995;332:1058–65.
[45] Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007;101:1266–73.
[46] Robinson P, Mirza M, Knott A, Abdulrazzak H, Willott R, Marston S, Watkins H, Redwood C. Alterations in thin filament regulation induced by a human cardiac troponin T mutant that causes dilated cardiomyopathy are distinct from those induced by troponin T mutants that cause hypertrophic cardiomyopathy. J Biol Chem. 2002;277 (43):40710–6.
[47] Wang X, Robbins J. Proteasomal and lysosomal protein degradation and heart disease. J Mol Cell Cardiol. 2014;71:16–24.
[48] Roncarati R, Viviani Anselmi C, Krawitz P, Lattanzi G, von Kodolitsch Y, Perrot A, di Pasquale E, Papa L, Portararo P, Columbaro M, Forni A, Faggian G, Condorelli G, Robinson PN. Doubly heterozygous LMNA and TTN mutations revealed by exome sequencing in a severe form of dilated cardiomyopathy. Eur J Hum Genet. 2013;21 (10):1105–11.
[49] Dhandapany PS, Sadayappan S, Xue Y, Powell GT, Rani DS, Nallari P, Rai TS, Khullar M, Soares P, Bahl A, Tharkan JM, Vaideeswar P, Rathinavel A, Narasimhan C, Ayapati DR, Ayub Q, Mehdi SQ, Oppenheimer S, Richards MB, Price AL, Patterson N, Reich D, Singh L, Tyler-Smith C, Thangaraj K. A common MYBPC3 (cardiac myosin binding protein C) variant associated with cardiomyopathies in South Asia. Nat Genet. 2009;41(2): 187–91.
[50] Helms AS, Davis FM, Coleman D, Bartolone SN, Glazier AA, Pagani F, Yob JM, Sadayappan S, Pedersen E, Lyons R, Westfall MV, Jones R, Russell MW, Day SM. Sarcomere mutation-specific expression patterns in human hypertrophic cardiomyopathy. Circ Cardiovasc Genet. 2014;7(4):434–43.
[51] Marston S, Copeland O, Jacques A, Livesey K, Tsang V, McKenna WJ, Jalilzadeh S, Carballo S, Redwood C, Watkins H. Evidence from human myectomy samples that MYBPC3 mutations cause hypertrophic cardiomyopathy through haploinsufficiency. Circ Res. 2009;105(3):219–22.
[52] van Dijk SJ, Dooijes D, dos Remedios C, Michels M, Lamers JM, Winegrad S, Schlossarek S, Carrier L, ten Cate FJ, Stienen GJ, van der Velden J. Cardiac myosin-binding protein C mutations and hypertrophic cardiomyopathy: haploinsufficiency, deranged phosphorylation, and cardiomyocyte dysfunction. Circulation. 2009;119 (11):1473–83.
[53] Wijnker PJ, Friedrich FW, Dutsch A, Reischmann S, Eder A, Mannhardt I, Mearini G, Eschenhagen T, van der Velden J, Carrier L. Comparison of the effects of a truncating and a missense MYBPC3 mutation on contractile parameters of engineered heart tissue. J Mol Cell Cardiol. 2016;97:82–92.
[54] Hassoun R, Budde H, Zhazykbayeva S, Herwig M, Sieme M, Delalat S, Mostafi N, Gömöri K, Tangos M, Jarkas M, Pabel S, Bruckmüller S, Skrygan M, Lódi M, Jaquet K, Sequeira V, Gambichler T, Remedios CD, Kovács Á, Mannherz HG, Mügge A, Sossalla S, Hamdani N. Stress activated signalling impaired protein quality control pathways in human hypertrophic cardiomyopathy. Int J Cardiol. 2021; 344:160–9.
[55] Bers DM. Cardiac excitation-contraction coupling. Nature. 2002;415:198–205.
[56] England PJ. Correlation between contraction and phosphorylation of the inhibitory subunit of troponin in perfused rat heart. FEBS Lett. 1975;50:57–60.
[57] Solaro RJ, Moir AJG, Perry SV. Phosphorylation of troponin I and the inotropic effect of adrenaline in the perfused rabbit heart. Nature. 1976;262:615–7.
[58] Lim MS, Walsh MP. Phosphorylation of skeletal and cardiac muscle C-proteins by the catalytic subunit of cAMP-dependent protein kinase. Biochem Cell Biol. 1986;64: 622–30.
[59] Gautel M, Zuffardi O, Freiburg A, Labeit S. Phosphorylation switches specific for the cardiac isoform of myosin binding protein-C: a modulator of cardiac contraction? EMBO J. 1995;14:1952–60.
[60] van Dijk SJ, Paalberends ER, Najafi A, Michels M, Sadayappan S, Carrier L, Boontje NM, Kuster DWD, van Slegtenhorst M, Dooijes D, dos Remedios C, ten Cate FJ, Stienen GJM, van der Velden J. Contractile dysfunction irrespective of the mutant protein in human hypertrophic cardiomyopathy with normal systolic function. Circ Heart Fail. 2012;5:36–46.
[61] Sirenko SG, Potter JD, Knollmann BC. Differential effect of troponin T mutations on the inotropic responsiveness of mouse hearts - role of myofilament Ca2+ sensitivity increase. J Physiol. 2006;575:201–13.
[62] Wijnker PJM, Foster DB, Tsao AL, Frazier AH, dos Remedios C, Murphy AM, Stienen GJM, van der Velden J. Impact of site-specific phosphorylation of the protein kinase a sites ser23 and ser24 of cardiac troponin in human cardiomyocytes. Am J Physiol. 2013;304:H260–8.
[63] van der Velden J, Papp Z, Zaremba R, Boontje NM, de Jong JW, Owen VJ, Burton PBJ, Goldmann P, Jaquet K, Stienen GJM. Increased Ca2+-sensitivity of the contractile apparatus in end-stage human heart failure results from altered phosphorylation of contractile proteins. Cardiovasc Res. 2003;57:37–47.
[64] Najafi A, Sequeira V, Helmes M, Bollen IAE, Goebel M, Regan JA, Carrier L, Kuster DWD, van der Velden J. Selective phosphorylation of PKA targets after β-adrenergic receptor stimulation impairs myofilament function in Mybpc3-targeted HCM mouse model. Cardiovas Res. 2016;110:200–14.
[65] Freeman K, Colon-Rivera C, Olsson MC, Moore RL, Weinberger HD, Grupp IL, Vikstrom KL, Iaccarino G, Koch WJ, Leinwand LA. Progression from hypertrophic to dilated cardiomyopathy in mice that express a mutant myosin transgene. Am J Physiol. 2001;280:H151–9.
[66] Prabhakar R, Boivin GP, Grupp IL, Hoit B, Arteaga G, Solaro JR, Wieczorek DF. A familial hypertrophic cardiomyopathy alpha-tropomyosin mutation causes severe cardiac hypertrophy and death in mice. J Mol Cell Cardiol. 2001;33:1815–28.
[67] Dweck D, Sanchez-Gonzalez MA, Chang AN, Dulce RA, Badger CD, Koutnik AP, Ruiz EL, Griffin B, Liang J, Kabbaj M, Fincham FD, Hare JM, Overton JM, Pinto JR. Long term ablation of protein kinase a (PKA)-mediated cardiac troponin I phosphorylation leads to excitation-contraction uncoupling and diastolic dysfunction in a knock-in mouse model of hypertrophic cardiomyopathy. J Biol Chem. 2014;289(33): 23097–111.
[68] Surdo NC, Berrera M, Koschinski A, Brescia M, Machado MR, Carr C, Wright P, Gorelik J, Morotti S, Grandi E, Bers DM, Pantano S, Zaccolo M. FRET biosensor uncovers cAMP nano-domains at beta-adrenergic targets that dictate precise tuning of cardiac contractility. Nat Commun. 2017;8:15031.
[69] Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. J Biol Chem. 1997;272(33):20313–6.
[70] Zhang W, Xiao S, Ahn D. Protein oxidation: basic principles and implications for meat quality. Crit Rev Food Sci Nutr. 2013;53:1191–201.
[71] Stathopoulou K, Wittig I, Heidler J, Piasecki A, Richter F, Diering S, van der Velden J, Buck F, Donzelli S, Schroder E, Wijnker PJ, Voigt N, Dobrev D, Sadayappan S, Eschenhagen T, Carrier L, Eaton P, Cuello F. S-glutathiolation impairs phosphoregulation and function of cardiac myosin-binding protein C in human heart failure. FASEB J. 2016;30(5):1849–64.
[72] Balderas-Villalobos J, Molina-Muñoz T, Mailloux-Salinas P, Bravo G, Carvajal K, Gómez-Viquez NL. Oxidative stress in cardiomyocytes contributes to decreased SERCA2a activity in rats with metabolic syndrome. Am J Physiol. 2013;305: H1344–53.
[73] Mekhfi H, Veksler V, Mateo P, Maupoil V, Rochette L, Ventura-Clapier R. Creatine kinase is the main target of reactive oxygen species in cardiac myofibrils. Circ Res. 1996; 78:1016–27.
[74] Cai Z, Yan LJ. Protein oxidative modifications: beneficial roles in disease and health. J Biochem Pharmacol Res. 2013;1(1):15–26.
[75] Wani R, Nagata A, Murray BW. Protein redox chemistry: post-translational cysteine modifications that regulate signal transduction and drug pharmacology. Front Pharmacol. 2014;5:224.
[76] Zhao T-J, Yan Y-B, Liu Y, Zhou H-M. The generation of the oxidized form of creatine kinase is a negative regulation on muscle creatine kinase*. Journal of Biological Chemistry. 2007;282(16):12022–9.
[77] Chen Y, Zhang Z, Hu F, Yang W, Yuan J, Cui J, Hao S, Hu J, Zhou Y, Qiao S. 17beta-estradiol prevents cardiac diastolic dysfunction by stimulating mitochondrial function: a preclinical study in a mouse model of a human hypertrophic cardiomyopathy mutation. J Steroid Biochem Mol Biol. 2015;147:92–102.
[78] Christiansen LB, Dela F, Koch J, Hansen CN, Leifsson PS, Yokota T. Impaired cardiac mitochondrial oxidative phosphorylation and enhanced mitochondrial oxidative stress in feline hypertrophic cardiomyopathy. Am J Physiol Heart Circ Physiol. 2015;308 (10):H1237–47.
[79] Lombardi R, Rodriguez G, Chen SN, Ripplinger CM, Li W, Chen J, Willerson JT, Betocchi S, Wickline SA, Efimov IR, Marian AJ. Resolution of established cardiac hypertrophy and fibrosis and prevention of systolic dysfunction in a transgenic rabbit model of human cardiomyopathy through thiol-sensitive mechanisms. Circulation. 2009;119(10):1398–407.
[80] Senthil V, Chen SN, Tsybouleva N, Halder T, Nagueh SF, Willerson JT, Roberts R, Marian AJ. Prevention of cardiac hypertrophy by atorvastatin in a transgenic rabbit model of human hypertrophic cardiomyopathy. Circ Res. 2005;97(3):285–92.
[81] Chimenti C, Scopelliti F, Vulpis E, Tafani M, Villanova L, Verardo R, De Paulis R, Russo MA, Frustaci A. Increased oxidative stress contributes to cardiomyocyte dysfunction and death in patients with fabry disease cardiomyopathy. Hum Pathol. 2015;46(11): 1760–8.
[82] Dimitrow PP, Undas A, Wolkow P, Tracz W, Dubiel JS. Enhanced oxidative stress in hypertrophic cardiomyopathy. Pharmacol Rep. 2009;61(3):491–5.
[83] Koda M, Takemura G, Okada H, Kanoh M, Maruyama R, Esaki M, Li Y, Miyata S, Kanamori H, Li L, Ogino A, Kondo T, Minatoguchi S, Fujiwara T, Fujiwara H. Nuclear hypertrophy reflects increased biosynthetic activities in myocytes of human hypertrophic hearts. Circ J. 2006;70(6):710–8.
[84] Nakamura K, Kusano KF, Matsubara H, Nakamura Y, Miura A, Nishii N, Banba K, Nagase S, Miyaji K, Morita H, Saito H, Emori T, Ohe T. Relationship between oxidative stress and systolic dysfunction in patients with hypertrophic cardiomyopathy. J Card Fail. 2005;11(2):117–23.
[85] Witjas-Paalberends ER, Ferrara C, Scellini B, Piroddi N, Montag J, Tesi C, Stienen GJM, Michels M, Ho CY, Kraft T, Poggesi C, van der Velden J. Faster cross-bridge detachment and increased tension cost in human hypertrophic cardiomyopathy with the R403Q MYH7 mutation. J Physiol. 2014;592:3257–72.
[86] Witjas-Paalberends ER, Güçlü A, Germans T, Knaapen P, Harms HJ, Vermeer AMC, Christiaans I, Wilde AAM, Dos Remedios C, Lammertsma AA, van Rossum AC, Stienen GJM, van Slegtenhorst M, Schinkel AF, Michels M, Ho CY, Poggesi C, van der Velden J.

Gene-specific increase in the energetic cost of contraction in hypertrophic cardiomyopathy caused by thick filament mutations. Cardiovasc Res. 2014;103:248–57.
[87] Spindler M, Saupe KW, Christe ME, Sweeney HL, Seidman CE, Seidman JG, Ingwall JS. Diastolic dysfunction and altered energetics in the alphaMHC403/+ mouse model of familial hypertrophic cardiomyopathy. J Clin Invest. 1998;101:1775–83.
[88] He H, Javadpour MM, Latif F, Tardiff JC, Ingwall JS. R-92L and R-92W mutations in cardiac troponin T lead to distinct energetic phenotypes in intact mouse hearts. Biophys J. 2007;93:1834–44.
[89] Huke S, Venkataraman R, Faggioni M, Bennuri S, Hwang HS, Baudenbacher F, Knollmann B. Focal energy deprivation underlies arrhythmia susceptibility in mice with calcium-sensitized myofilaments. Circ Res. 2013;112:1334–44.
[90] Crilley JG, Boehm EA, Blair E, Rajagopalan B, Blamire AM, Styles P, McKenna WJ, Ostman-Smith I, Clarke K, Watkins H. Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. J Am Coll Cardiol. 2003;41(10):1776–82.
[91] Güçlü A, Knaapen P, Harms Hendrik J, Parbhudayal Rahana Y, Michels M, Lammertsma Adriaan A, van Rossum Albert C, Germans T, van der Velden J. Disease stage-dependent changes in cardiac contractile performance and oxygen utilization underlie reduced myocardial efficiency in human inherited hypertrophic cardiomyopathy. Circ Cardiovasc Imag. 2017;10(5):e005604.
[92] Javadpour MM, Tardiff JC, Pinz I, Ingwall JS. Decreased energetics in murine hearts bearing the R92Q mutation in cardiac troponin T. J Clin Invest. 2003;112:768–75.
[93] Ashrafian H, Redwood C, Blair E, Watkins H. Hypertrophic cardiomyopathy:a paradigm for myocardial energy depletion. Trends Genet. 2003;19:263–8.
[94] Neubauer S, Horn M, Pabst T, Gödde M, Lübke D, Jilling B, Hahn D, Ertl G. Contributions of 31P-magnetic resonance spectroscopy to the understanding of dilated heart muscle disease. Eur Heart J. 1995;16:115–8.
[95] Greenhaff PL. The creatine-phosphocreatine system: there's more than one song in its repertoire. J Physiol. 2001;537:657.
[96] Smith CS, Bottomley PA, Schulman SP, Gerstenblith G, Weiss RG. Altered creatine kinase adenosine triphosphate kinetics in failing hypertrophied human myocardium. Circulation. 2006;114:1151–8.
[97] Abraham MR, Bottomley PA, Dimaano VL, Pinheiro A, Steinberg A, Traill TA, Abraham TP, Weiss RG. Creatine kinase adenosine triphosphate and phosphocreatine energy supply in a single kindred of patients with hypertrophic cardiomyopathy. Am J Cardiol. 2013;112:861–6.
[98] Miller T, Szczesna D, Housmans PR, Zhao J, de Freitas F, Gomes AV, Culbreath L, McCue J, Wang Y, Xu Y, Kerrick WG, Potter JD. Abnormal contractile function in transgenic mice expressing a familial hypertrophic cardiomyopathy-linked troponin T (I79N) mutation. J Biol Chem. 2001;276(6):3743–55.
[99] Vignier N, Schlossarek S, Fraysse B, Mearini G, Kramer E, Pointu H, Mougenot N, Guiard J, Reimer R, Hohenberg H, Schwartz K, Vernet M, Eschenhagen T, Carrier L. Nonsense-mediated mRNA decay and ubiquitin-proteasome system regulate cardiac myosin-binding protein C mutant levels in cardiomyopathic mice. Circ Res. 2009;105(3):239–48.
[100] Knollmann BC, Blatt SA, Horton K, de Freitas F, Miller T, Bell M, Housmans PR, Weissman NJ, Morad M, Potter JD. Inotropic stimulation induces cardiac dysfunction in transgenic mice expressing a troponin T (I79N) mutation linked to familial hypertrophic cardiomyopathy. J Biol Chem. 2001;276(13):10039–48.
[101] Baudenbacher F, Schober T, Pinto JR, Sidorov VY, Hilliard F, Solaro RJ, Potter JD, Knollmann BC. Myofilament Ca2+ sensitization causes susceptibility to cardiac arrhythmia in mice. J Clin Invest. 2008;118(12):3893–903.
[102] Lucas DT, Aryal P, Szweda LI, Koch WJ, Leinwand LA. Alterations in mitochondrial function in a mouse model of hypertrophic cardiomyopathy. Am J Physiol. 2003;284:H575–83.
[103] Unno K, Isobe S, Izawa H, Cheng XW, Kobayashi M, Hirashiki A, Yamada T, Harada K, Ohshima S, Noda A, Nagata K, Kato K, Yokota M, Murohara T. Relation of functional and morphological changes in mitochondria to myocardial contractile and relaxation reserves in asymptomatic to mildly symptomatic patients with hypertrophic cardiomyopathy. Cardiovasc Res. 2009;30:1853–62.
[104] Nollet EE, Duursma I, Rozenbaum A, Eggelbusch M, Wüst RCI, Schoonvelde SAC, et al. Mitochondrial dysfunction in human hypertrophic cardiomyopathy is linked to cardiomyocyte architecture disruption and corrected by improving NADH-driven mitochondrial respiration. Eur Heart J. 2023;44:1170–85.
[105] Sequeira V, Najafi A, McConnell M, Fowler ED, Bollen IAE, Wüst RCI, Helmes M, White E, Stienen GJM, Tardiff J, Kuster DWD, van der Velden J, Remedios CD. Synergistic role of ADP and Ca2+ in diastolic myocardial stiffness. J Physiol. 2015;593:3899–916.
[106] Bertero E, Maack C. Calcium signaling and reactive oxygen species in mitochondria. Circ Res. 2018;122:1460–78.
[107] Nickel Alexander G, von Hardenberg A, Hohl M, Löffler Joachim R, Kohlhaas M, Becker J, Reil J-C, Kazakov A, Bonnekoh J, Stadelmaier M, Puhl S-L, Wagner M, Bogeski I, Cortassa S, Kappl R, Pasieka B, Lafontaine M, Roy C, Lancaster D, Blacker Thomas S, Hall Andrew R, Duchen Michael R, Kästner L, Lipp P, Zeller T, Müller C, Knopp A, Laufs U, Böhm M, Hoth M, Maack C. Reversal of mitochondrial transhydrogenase causes oxidative stress in heart failure. Cell Metab. 2015;22:472–84.
[108] Marian AJ, Senthil V, Chen SN, Lombardi R. AnAntifibrotic effects of antioxidant N-acetylcysteine in a mouse model of human hypertrophic cardiomyopathy mutation. J Am Coll Cardio. 2006;47:827–34.
[109] Lombardi R, Rodriguez G, Chen SN, Ripplinger CM, Li W, Chen J, Willerson JT, Betocchi S, Wickline SA, Efimov IR, Marian AJ. Resolution of established cardiac hypertrophy and fibrosis and prevention of systolic dysfunction in a transgenic rabbit model of human cardiomyopathy through thiol-sensitive mechanisms. Circulation. 2009;119:1398–407.
[110] Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, Bartlett RK, Lowe JS, O'Donnell SE, Aykin-Burns N, Zimmerman MC, Zimmerman K, Ham AJ, Weiss RM, Spitz DR, Shea MA, Colbran RJ, Mohler PJ, Anderson ME. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. Cell. 2008;133:462–74.
[111] Wagner S, Ruff HM, Weber SL, Bellmann S, Sowa T, Schulte T, Anderson ME, Grandi E, Bers DM, Backs J, Belardinelli L, Maier LS. Reactive oxygen species-activated Ca/calmodulin kinase IIδ is required for late I(Na) augmentation leading to cellular Na and Ca overload. Circ Res. 2011;108:555–65.
[112] Ago T, Liu T, Zhai P, Chen W, Li H, Molkentin JD, Vatner SF, Sadoshima J. A redox-dependent pathway for regulating class II HDACs and cardiac hypertrophy. Cell. 2008;133:978–93.
[113] Helms AS, Alvarado FJ, Yob J, Tang VT, Pagani F, Russell MW, Valdivia HH, Day SM. Genotype-dependent and -independent calcium signaling dysregulation in human hypertrophic cardiomyopathy. Circulation. 2016;134:1738–48.
[114] Torres M, Forman HJ. Redox signaling and the MAP kinase pathways. Biofactors. 2003;17(1–4):287–96.
[115] Davis J, Davis LC, Correll Robert N, Makarewich Catherine A, Schwanekamp Jennifer A, Moussavi-Harami F, Wang D, York Allen J, Wu H, Houser Steven R, Seidman Christine E, Seidman Jonathan G, Regnier M, Metzger Joseph M, Wu Joseph C, Molkentin Jeffery D. A tension-based model distinguishes hypertrophic versus dilated cardiomyopathy. Cell. 2016;165:1147–59.
[116] Bayrak F, Komurcu-Bayrak E, Mutlu B, Kahveci G, Basaran Y, Erginel-Unaltuna N. Ventricular pre-excitation and cardiac hypertrophy mimicking hypertrophic cardiomyopathy in a turkish family with a novel PRKAG2 mutation. Eur J Heart Fail. 2006;8(7):712–5.
[117] Banerjee SK, McGaffin KR, Huang XN, Ahmad F. Activation of cardiac hypertrophic signaling pathways in a transgenic mouse with the human PRKAG2 Thr400Asn mutation. Biochim Biophys Acta. 1802;2010:284–91.
[118] Lee PJ, Deanfield JE, Burch M, Baig K, McKenna WJ, Leonard JV. Comparison of the functional significance of left ventricular hypertrophy in hypertrophic cardiomyopathy and glycogenosis type III. Am J Cardiol. 1997;79:834–8.
[119] Browne GJ, Finn SG, Proud CG. Stimulation of the AMP-activated protein kinase leads to activation of eukaryotic elongation factor 2 kinase and to its phosphorylation at a novel site, serine 398. J Biol Chem. 2004;279:12220–31.
[120] Chan AYM, Soltys C-LM, Young ME, Proud CG, Dyck JRB. Activation of AMP-activated protein kinase inhibits protein synthesis associated with hypertrophy in the cardiac myocyte. J Biol Chem. 2004;279:32771–9.
[121] Carling D, Grahame Hardie D. The substrate and sequence specificity of the AMP-activated protein kinase. Phosphorylation of glycogen synthase and phosphorylase kinase. Biochim Biophys Acta. 1989;1012:81–6.
[122] Halse R, Fryer LGD, McCormack JG, Carling D, Yeaman SJ. Regulation of glycogen synthase by glucose and glycogen: a possible role for AMP-activated protein kinase. Diabetes. 2003;52:9–15.
[123] Maron BJ, Ferrans VJ. Intramitochondrial glycogen deposits in hypertrophied human myocardium. J Mol Cell Cardiol. 1975;7(9):699–702.
[124] Maron BJ, Ferrans VJ, Henry WL, Clark CE, Redwood DR, Roberts WC, Morrow AG, Epstein SE. Differences in distribution of myocardial abnormalities in patients with obstructive and nonobstructive asymmetric septal hypertrophy (ASH). Circulation. 1974;50(3):436–46.
[125] Maron BJ, Ferrans VJ, Roberts WC. Ultrastructural features of degenerated cardiac muscle cells in patients with cardiac hypertrophy. Am J Pathol. 1975;79(3):387–434.
[126] Schuldt M, Pei J, Harakalova M, Dorsch LM, Schlossarek S, Mokry M, Knol JC, Pham TV, Schelfhorst T, Piersma SR, Dos Remedios C, Dalinghaus M, Michels M, Asselbergs FW, Moutin MJ, Carrier L, Jimenez CR, van der Velden J, Kuster DWD. Proteomic and functional studies reveal detyrosinated tubulin as treatment target in sarcomere mutation-induced hypertrophic cardiomyopathy. Circ Heart Fail. 2021;14(1):e007022.
[127] Fassett JT, Hu X, Xu X, Lu Z, Zhang P, Chen Y, Bache RJ. AMPK attenuates microtubule proliferation in cardiac hypertrophy. Am J Physiol. 2013;304:H749–58.
[128] Calamaras TD, Lee C, Lan F, Ido Y, Siwik DA, Colucci WS. The lipid peroxidation product 4-hydroxy-trans-2-nonenal causes protein synthesis in cardiac myocytes via activated mTORC1–p70S6K–RPS6 signaling. Free Radic Biol Med. 2015;82:137–46.
[129] Baudenbacher F, Schober T, Pinto JR, Sidorov VY, Hilliard F, Solaro RJ, Potter JD, Knollmann BC. Myofilament Ca2+ sensitization causes susceptibility to cardiac arrhythmia in mice. J Clin Inv. 2008;118:3893–903.
[130] Coppini R, Ferrantini C, Yao L, Fan P, Del Lungo M, Stillitano F, Sartiani L, Tosi B, Suffredini S, Tesi C, Yacoub M, Olivotto I, Belardinelli L, Poggesi C, Cerbai E, Mugelli A. Late sodium current inhibition reverses electromechanical dysfunction in human hypertrophic cardiomyopathy. Circulation. 2013;27:575–84.
[131] Allen DG, Orchard CH. Myocardial contractile function during ischemia and hypoxia. Circ Res. 1987;60:153–68.
[132] Kléber AG. Gap junctions and conduction of cardiac excitation. Heart Rhythm. 2011;8:1981–4.
[133] Beardslee MA, Lerner DL, Tadros PN, Laing JG, Beyer EC, Yamada KA, Kléber AG, Schuessler RB, Saffitz JE. Dephosphorylation and intracellular redistribution of ventricular Connexin43 during electrical uncoupling induced by ischemia. Circ Res. 2000;87(8):656–62.
[134] Turner MS, Haywood GA, Andreka P, You L, Martin PE, Evans WH, Webster KA, Bishopric NH. Reversible connexin 43 dephosphorylation during hypoxia and reoxygenation is linked to cellular ATP levels. Circ Res. 2004;95:726–33.
[135] Hegyi B, Pölönen RP, Hellgren KT, Ko CY, Ginsburg KS, Bossuyt J, Mercola M, Bers DM. Cardiomyocyte Na+ and Ca2+ mishandling drives vicious cycle involving CaMKII, ROS, and ryanodine receptors. Basic Res Cardiol. 2021;116:58.
[136] Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof P, Maier SK, Zhang T, Hasenfuss G, Brown JH, Bers DM, Maier LS. Ca2+/calmodulin-dependent protein kinase II regulates cardiac Na+ channels. J Clin Invest. 2006;116:3127–38.
[137] Coppini R, Ferrantini C, Yao L, Fan P, Del Lungo M, Stillitano F, Sartiani L, Tosi B, Suffredini S, Tesi C, Yacoub M, Olivotto I, Belardinelli L, Poggesi C, Cerbai E,

Mugelli A. Late sodium current inhibition reverses electromechanical dysfunction in human hypertrophic cardiomyopathy. Circulation. 2013;127(5):575–84.
[138] Lehman SJ, Tal-Grinspan L, Lynn ML, Strom J, Benitez GE, Anderson ME, Tardiff JC. Chronic calmodulin-kinase II activation drives disease progression in mutation-specific hypertrophic cardiomyopathy. Circulation. 2019;139:1517–29.
[139] Morris TE, Sulakhe PV. Sarcoplasmic reticulum Ca2+-pump dysfunction in rat cardiomyocytes briefly exposed to hydroxyl radicals. Free Radic Biol Med. 1997;22: 37–47.
[140] Wagner S, Rokita AG, Anderson ME, Maier LS. Redox regulation of sodium and calcium handling. Antioxid Redox Signal. 2013;18:1063–77.
[141] Nikolaienko R, Bovo E, Zima AV. Redox dependent modifications of ryanodine receptor: basic mechanisms and implications in heart diseases. Front Physiol. 2018.;9.
[142] Yang KC, Kyle JW, Makielski JC, Dudley Jr SC. Mechanisms of sudden cardiac death: oxidants and metabolism. Circ Res. 2015;116(12):1937–55.
[143] Yan Y, Liu J, Wei C, Li K, Xie W, Wang Y, Cheng H. Bidirectional regulation of Ca2+ sparks by mitochondria-derived reactive oxygen species in cardiac myocytes. Cardiovasc Res. 2008;77:432–41.
[144] Kohlhaas M, Parikh S, Sequeira V, Dietl A, Richter O, Bay J, Tian Q, Friedrich FW, Flenner F, Nickel G, Alscher F, Venkataraman R, Baudenbacher FJ, Bertero E, Leticia Prates Roma, Kazakow A, Hohl M, Laufs U, Hoth M, Böhm M, Lipp P, Carrier L, Eschenhagen T, Knollmann BC, MC. Mitochondrial redox mismatch and reactive oxygen species cause arrhythmias in hypertrophic cardiomyopathy. In: Cardiol CR, editor. Jahrestagung der Deutsche Gesellschaft für Kardiologie- Herz- und Kreislaufforschung vom 24. bis 27. April 2019 in Mannheim, Mannheim, 85. ; 2019.
[145] Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. Physiol Rev. 2005;85(3):1093–129.
[146] Lopaschuk GD, Ussher JR, Folmes CDL, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. Physiol Rev. 2010;90(1):207–58.
[147] Fontaine E, Leverve X. Basics in clinical nutrition: metabolic response to hypoxia. European e. 2008;3(6):e285–8.
[148] Giuseppe MCR, Massimo F, Giuseppe C, Giuseppe B. Cardiac metabolism in myocardial ischemia. Curr Pharm Des. 2008;14:2551–62.
[149] Neely JR, Morgan HE. Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Annu Rev Physiol. 1974;36:413–59.
[150] Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1963; 1(7285):785–9.
[151] Li J, Kemp BA, Howell NL, Massey J, Mińczuk K, Huang Q, Chordia MD, Roy RJ, Patrie JT, Davogustto GE, Kramer CM, Epstein FH, Carey RM, Taegtmeyer H, Keller SR, Kundu BK. Metabolic changes in spontaneously hypertensive rat hearts precede cardiac dysfunction and left ventricular hypertrophy. J Am Heart Assoc. 2019;8:e010926.
[152] Ritterhoff J, Young S, Villet O, Shao D, Neto FC, Bettcher LF, Hsu YA, Kolwicz Jr SC, Raftery D, Tian R. Metabolic remodeling promotes cardiac hypertrophy by directing glucose to aspartate biosynthesis. Circ Res. 2020;126:182–96.
[153] Hamirani YS, Kundu BK, Zhong M, McBride A, Li Y, Davogustto GE, Taegtmeyer H, Bourque JM. Noninvasive detection of early metabolic left ventricular remodeling in systemic hypertension. Cardiology. 2016;133:157–62.
[154] Aoyama R, Takano H, Kobayashi Y, Kitamura M, Asai K, Amano Y, Kumita S-I, Shimizu W. Evaluation of myocardial glucose metabolism in hypertrophic cardiomyopathy using 18F-fluorodeoxyglucose positron emission tomography. PLoS ONE. 2017;12 (11):e0188479.
[155] Olivotto I, Girolami F, Sciagrà R, Ackerman MJ, Sotgia B, Bos JM, Nistri S, Sgalambro A, Grifoni C, Torricelli F, Camici PG, Cecchi F. Microvascular function is selectively impaired in patients with hypertrophic cardiomyopathy and sarcomere myofilament gene mutations. J Am Coll Cardiol. 2011;58:839–48.
[156] Camici PG, Olivotto I, Rimoldi OE. The coronary circulation and blood flow in left ventricular hypertrophy. J Mol Cell Cardiol. 2012;52:857–64.
[157] Petersen SE, Jerosch-Herold M, Hudsmith LE, Robson MD, Francis JM, Doll HA, Selvanayagam JB, Neubauer S, Watkins H. Evidence for microvascular dysfunction in hypertrophic cardiomyopathy: new insights from multiparametric magnetic resonance imaging. Circulation. 2007;115(18):2418–25.
[158] Olivotto I, d'Amati G, Basso C, Van Rossum A, Patten M, Emdin M, Pinto Y, Tomberli B, Camici PG, Michels M. Defining phenotypes and disease progression in sarcomeric cardiomyopathies: contemporary role of clinical investigations. Cardiovasc Res. 2015;105 (4):409–23.
[159] Tanaka T, Sohmiya K, Kawamura K. Is CD36 deficiency an etiology of hereditary hypertrophic cardiomyopathy? J Mol Cell Cardio. 1997;29(1):121–7.
[160] Lahey R, Wang X, Carley AN, Lewandowski ED. Dietary fat supply to failing hearts determines dynamic lipid signaling for nuclear receptor activation and oxidation of stored triglyceride. Circulation. 2014;130(20):1790–9.
[161] Bertero E, Maack C. Metabolic remodelling in heart failure. Nat Rev Cardiol. 2018;15 (8):457–70.
[162] Chung H, Kim J-Y, Min P-K, Yoon YW, Lee BK, Hong B-K, Rim S-J, Kwon HM, Choi E-Y. Different contribution of sarcomere and mitochondrial related gene mutations to hypertrophic cardiomyopathy. J Am Coll Cardiol. 2018;71(11 Supplement):A901.
[163] Batzner A, Schäfers HJ, Borisov KV, Seggewiß H. Hypertrophic obstructive cardiomyopathy. Dtsch Arztebl Int. 2019;116:47–53.
[164] Steendijk P, Meliga E, Valgimigli M, Ten Cate FJ, Serruys PW. Acute effects of alcohol septal ablation on systolic and diastolic left ventricular function in patients with hypertrophic obstructive cardiomyopathy. Heart. 2008;94(10):1318–22.
[165] Ommen SR, Mital S, Burke MA, Day SM, Deswal A, Elliott P, Evanovich LL, Hung J, Joglar JA, Kantor P, Kimmelstiel C, Kittleson M, Link MS, Maron MS, Martinez MW, Miyake CY, Schaff HV, Semsarian C, Sorajja P. 2020 AHA/ACC guideline for the diagnosis and treatment of patients with hypertrophic cardiomyopathy: a report of the american College of Cardiology/American Heart Association joint committee on clinical practice guidelines. Circulation. 2020;142(25):e558–631.
[166] Cecchi F, Olivotto I, Gistri R, Lorenzoni R, Chiriatti G, Camici PG. Coronary microvascular dysfunction and prognosis in hypertrophic cardiomyopathy. N Engl J Med. 2003; 349(11):1027–35.
[167] Davies JE, Whinnett ZI, Francis DP, Manisty CH, Aguado-Sierra J, Willson K, Foale RA, Malik IS, Hughes AD, Parker KH, Mayet J. Evidence of a dominant backward-propagating "suction" wave responsible for diastolic coronary filling in humans, attenuated in left ventricular hypertrophy. Circulation. 2006;113:1768–78.
[168] Eskerud I, Gerdts E, Larsen TH, Lonnebakken MT. Left ventricular hypertrophy contributes to myocardial ischemia in non-obstructive coronary artery disease (the MicroCAD study). Int J Cardiol. 2019;286:1–6.
[169] Hughes S. The pathology of hypertrophic cardiomyopathy. Histopathology. 2004;44 (5):412–27.
[170] Maron BJ, Wolfson JK, Epstein SE, Roberts WC. Intramural ("small vessel") coronary artery disease in hypertrophic cardiomyopathy. J Am Coll Cardiol. 1986;8(3):545–57.
[171] Louie EK, Maron BJ. Hypertrophic Cardiomyopathy With extreme increase in left ventricular wall thickness: functional and morphologic features and clinical significance. Elsevier; 1986. p. 57–65.
[172] Lamke GT, Allen RD, Edwards WD, Tazelaar HD, Danielson GK. Surgical pathology of subaortic septal myectomy associated with hypertrophic cardiomyopathy. A study of 204 cases (1996–2000). Cardiovasc Pathol. 2003;12(3):149–58.
[173] Langa P, Marszalek RJ, Warren CM, Chowdhury SK, Halas M, Batra A, Rafael-Clyke K, Bacon A, Goldspink PH, Solaro RJ, Wolska BM. Altered coronary artery function, arteriogenesis and endothelial YAP signaling in postnatal hypertrophic cardiomyopathy. Front Physiol. 2023.;14.
[174] Wijnker PJM, Sequeira V, Foster DB, Li Y, dos Remedios CG, Murphy AM, Stienen GJM, van der Velden J. Length-dependent activation is modulated by cardiac troponin I bisphosphorylation at Ser23 and Ser24 but not by Thr143 phosphorylation. Am J Physiol. 2014;306:H1171–81.
[175] Deidda M, Noto A, Pasqualucci D, Fattuoni C, Barberini L, Piras C, Bassareo PP, Porcu M, Mercuro G, Dessalvi CC. The echocardiographic parameters of systolic function are associated with specific metabolomic fingerprints in obstructive and non-obstructive hypertrophic cardiomyopathy. Metabolites. 2021.;11(11).
[176] Aubert G, Martin Ola J, Horton Julie L, Lai L, Vega Rick B, Leone Teresa C, Koves T, Gardell Stephen J, Krüger M, Hoppel Charles L, Lewandowski ED, Crawford Peter A, Muoio Deborah M, Kelly Daniel P. The failing heart relies on ketone bodies as a fuel. Circulation. 2016;133(8):698–705.
[177] Bedi Kenneth C, Snyder Nathaniel W, Brandimarto J, Aziz M, Mesaros C, Worth Andrew J, Wang Linda L, Javaheri A, Blair Ian A, Margulies Kenneth B, Rame JE. Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. Circulation. 2016;133(8):706–16.
[178] Voros G, Ector J, Garweg C, Droogne W, Van Cleemput J, Peersman N, Vermeersch P, Janssens S. Increased cardiac uptake of ketone bodies and free fatty acids in human heart failure and hypertrophic left ventricular remodeling. Circulation. 2018;11(12): e004953.
[179] Shimazu T, Hirschey MD, Newman J, He W, Shirakawa K, Le Moan N, Grueter CA, Lim H, Saunders LR, Stevens RD, Newgard CB, Farese RV, de Cabo R, Ulrich S, Akassoglou K, Verdin E. Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. Science. 2013;339(6116):211–4.
[180] Ooi JY, Tuano NK, Rafehi H, Gao XM, Ziemann M, Du XJ, El-Osta A. HDAC inhibition attenuates cardiac hypertrophy by acetylation and deacetylation of target genes. Epigenetics. 2015;10(5):418–30.
[181] Youm Y-H, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, D'Agostino D, Planavsky N, Lupfer C, Kanneganti TD, Kang S, Horvath TL, Fahmy TM, Crawford PA, Biragyn A, Alnemri E, Dixit VD. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome–mediated inflammatory disease. Nat Med. 2015;21(3):263–9.
[182] Butler J, Packer M, Filippatos G, Ferreira JP, Zeller C, Schnee J, Brueckmann M, Pocock SJ, Zannad F, Anker SD. Effect of empagliflozin in patients with heart failure across the spectrum of left ventricular ejection fraction. Eur Heart J. 2022;43(5):416–26.
[183] Osataphan S, Macchi C, Singhal G, Chimene-Weiss J, Sales V, Kozuka C, Dreyfuss JM, Pan H, Tangcharoenpaisan Y, Morningstar J, Gerszten R, Patti ME. SGLT2 inhibition reprograms systemic metabolism via FGF21-dependent and -independent mechanisms. JCI Insight. 2019.;4(5).
[184] Abozguia K, Elliott P, McKenna W, Phan TT, Nallur-Shivu G, Ahmed I, Maher AR, Kaur K, Taylor J, Henning A, Ashrafian H, Watkins H, Frenneaux M. Metabolic modulator perhexiline corrects energy deficiency and improves exercise capacity in symptomatic hypertrophic cardiomyopathy. Circulation. 2010;122:1562–9.
[185] Gehmlich K, Dodd M, William Allwood J, Kelly M, Bellahcene M, Lad H, Stockenhuber A, Hooper C, Ashrafian H, Redwood C, Carrier L, Dunn W. Changes in the cardiac metabolome caused by perhexiline treatment in a mouse model of hypertrophic cardiomyopathy. Mol Biosyst. 2014;11:9.
[186] Gatto Jr GJ, Ao Z, Kearse MG, Zhou M, Morales CR, Daniels E, Bradley BT, Goserud MT, Goodman KB, Douglas SA, Harpel MR, Johns DG. NADPH oxidase-dependent and -independent mechanisms of reported inhibitors of reactive oxygen generation. J Enzyme Inhib Med Chem. 2013;28(1):95–104.
[187] Kennedy JA, Beck-Oldach K, McFadden-Lewis K, Murphy GA, Wong YW, Zhang Y, Horowitz JD. Effect of the anti-anginal agent, perhexiline, on neutrophil, valvular and vascular superoxide formation. Eur J Pharmacol. 2006;531(1–3):13–9.
[188] Rayner JJ, Peterzan MA, Watson WD, Clarke WT, Neubauer S, Rodgers CT, Rider OJ. Myocardial energetics in obesity: enhanced ATP delivery through creatine kinase with blunted stress response. Circulation. 2020;141(14):1152–63.
[189] Marian AJ, Senthil V, Chen SN, Lombardi R. Antifibrotic effects of antioxidant N-acetylcysteine in a mouse model of human hypertrophic cardiomyopathy mutation. J Am Coll Cardiol. 2006;47(4):827–34.
[190] Slone S, Anthony SR, Wu X, Benoit JB, Aube J, Xu L, Tranter M. Activation of HuR downstream of p38 MAPK promotes cardiomyocyte hypertrophy. Cell Signal. 2016; 28(11):1735–41.

[191] Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med. 1989;6(6):593–7.
[192] Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci. 2003;60(1):6–20.
[193] Osman C, Voelker DR, Langer T. Making heads or tails of phospholipids in mitochondria. J Cell Biol. 2011;192:7–16.
[194] Colbeau A, Nachbaur J, Vignais PM. Enzymac characterization and lipid composition of rat liver subcellular membranes. Biochim Biophys Acta. 1971;249:462–92.
[195] Hovius R, Lambrechts H, Nicolay K, de Kruijff B. Improved methods to isolate and subfractionate rat liver mitochondria. Lipid composition of the inner and outer membrane. Biochim Biophys Acta. 1990;1021:217–26.
[196] Gebert N, Joshi AS, Kutik S, Becker T, McKenzie M, Guan XL, Mooga VP, Stroud DA, Kulkarni G, Wenk MR, Rehling P, Meisinger C, Ryan MT, Wiedemann N, Greenberg ML, Pfanner N. Mitochondrial cardiolipin involved in outer-membrane protein biogenesis: implications for Barth syndrome. Curr Biol. 2009;19(24):2133–9.
[197] Yin H, Zhu M. Free radical oxidation of cardiolipin: chemical mechanisms, detection and implication in apoptosis, mitochondrial dysfunction and human diseases. Free Radic Res. 2012;46(8):959–74.
[198] Szeto HH. First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. Br J Pharmacol. 2014;171:2029–50.
[199] Szeto HH, Liu S. Cardiolipin-targeted peptides rejuvenate mitochondrial function, remodel mitochondria, and promote tissue regeneration during aging. Arch Biochem Biophys. 2018;660:137–48.
[200] Birk AV, Chao WM, Bracken C, Warren JD, Szeto HH. Targeting mitochondrial cardiolipin and the cytochrome c/cardiolipin complex to promote electron transport and optimize mitochondrial ATP synthesis. Br J Pharmacol. 2014;171(8):2017–28.
[201] Birk AV, Liu S, Soong Y, Mills W, Singh P, Warren JD, Seshan SV, Pardee JD, Szeto HH. The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin. J Am Soc Nephrol. 2013;24:1250–61.
[202] Langsjoen PH, Langsjoen A, Willis R, Folkers K. Treatment of hypertrophic cardiomyopathy with coenzyme Q10. Mol Aspects Med. 1997;18(Suppl):S145–51.
[203] Adarsh K, Kaur H, Mohan V. Coenzyme Q10 (CoQ10) in isolated diastolic heart failure in hypertrophic cardiomyopathy (HCM). Biofactors. 2008;32(1–4):145–9.
[204] Abdellatif M, Trummer-Herbst V, Koser F, Durand S, Adão R, Vasques-Nóvoa F, Freundt JK, Voglhuber J, Pricolo MR, Kasa M, Türk C, Aprahamian F, Herrero-Galán E, Hofer SJ, Pendl T, Rech L, Kargl J, Anto-Michel N, Ljubojevic-Holzer S, Schipke J, Brandenberger C, Auer M, Schreiber R, Koyani CN, Heinemann A, Zirlik A, Schmidt A, von Lewinski D, Scherr D, Rainer PP, von Maltzahn J, Mühlfeld C, Krüger M, Frank S, Madeo F, Eisenberg T, Prokesch A, Leite-Moreira AF, Lourenço AP, Alegre-Cebollada J, Kiechl S, Linke WA, Kroemer G, Sedej S. Nicotinamide for the treatment of heart failure with preserved ejection fraction. Sci Transl Med. 2021.;13(580).
[205] Diguet N, Trammell SAJ, Tannous C, Deloux R, Piquereau J, Mougenot N, Gouge A, Gressette M, Manoury B, Blanc J, Breton M, Decaux JF, Lavery GG, Baczkó I, Zoll J, Garnier A, Li Z, Brenner C, Mericskay M. Nicotinamide riboside preserves cardiac function in a mouse model of dilated cardiomyopathy. Circulation. 2018;137(21):2256–73.
[206] Green EM, Wakimoto H, Anderson RL, Evanchik MJ, Gorham JM, Harrison BC, Henze M, Kawas R, Oslob JD, Rodriguez HM, Song Y, Wan W, Leinwand LA, Spudich JA, McDowell RS, Seidman JG, Seidman CE. A small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice. Science. 2016;351:617–21.
[207] Anderson RL, Trivedi DV, Sarkar SS, Henze M, Ma W, Gong H, Rogers CS, Gorham JM, Wong FL, Morck MM, Seidman JG, Ruppel KM, Irving TC, Cooke R, Green EM, Spudich JA. Deciphering the super relaxed state of human β-cardiac myosin and the mode of action of mavacamten from myosin molecules to muscle fibers. Proc Natl Acad Sci. 2018;115:E8143–52.
[208] Hegde SM, Lester SJ, Solomon SD, Michels M, Elliott PM, Nagueh SF, Choudhury L, Zemanek D, Zwas DR, Jacoby D, Wang A, Ho CY, Li W, Sehnert AJ, Olivotto I, Abraham TP. Effect of mavacamten on echocardiographic features in symptomatic patients with obstructive hypertrophic cardiomyopathy. J Am Coll Cardiol. 2021;78(25):2518–32.
[209] Saberi S, Cardim N, Yamani M, Schulz-Menger J, Li W, Florea V, Sehnert AJ, Kwong RY, Jerosch-Herold M, Masri A, Owens A, Lakdawala NK, Kramer CM, Sherrid M, Seidler T, Wang A, Sedaghat-Hamedani F, Meder B, Havakuk O, Jacoby D. Mavacamten favorably impacts cardiac structure in obstructive hypertrophic cardiomyopathy: EXPLORER-HCM cardiac magnetic resonance substudy analysis. Circulation. 2021;143(6):606–8.
[210] Ho CY, Mealiffe ME, Bach RG, Bhattacharya M, Choudhury L, Edelberg JM, Hegde SM, Jacoby D, Lakdawala NK, Lester SJ, Ma Y, Marian AJ, Nagueh SF, Owens A, Rader F, Saberi S, Sehnert AJ, Sherrid MV, Solomon SD, Wang A, Wever-Pinzon O, Wong TC, Heitner SB. Evaluation of mavacamten in symptomatic patients with nonobstructive hypertrophic cardiomyopathy. J Am Coll Cardiol. 2020;75(21):2649–60.
[211] Olivotto I, Oreziak A, Barriales-Villa R, Abraham TP, Masri A, Garcia-Pavia P, Saberi S, Lakdawala NK, Wheeler MT, Owens A, Kubanek M, Wojakowski W, Jensen MK, Gimeno-Blanes J, Afshar K, Myers J, Hegde SM, Solomon SD, Sehnert AJ, Zhang D, Li W, Bhattacharya M, Edelberg JM, Waldman CB, Lester SJ, Wang A, Ho CY, Jacoby D. Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2020;396(10253):759–69.
[212] Lehman SJ, Crocini C, Leinwand LA. Targeting the sarcomere in inherited cardiomyopathies. Nat Rev Cardiol. 2022;19:353–63.
[213] Olson EN. Toward the correction of muscular dystrophy by gene editing. Proceedings of the National Academy of Sciences. 2021;118(22):e2004840117.
[214] Chai AC, Cui M, Chemello F, Li H, Chen K, Tan W, Atmanli A, McAnally JR, Zhang Y, Xu L, Liu N, Bassel-Duby R, Olson EN. Base editing correction of hypertrophic cardiomyopathy in human cardiomyocytes and humanized mice. Nat Med. 2023;29(2):401–11.
[215] Reichart D, Newby GA, Wakimoto H, Lun M, Gorham JM, Curran JJ, Raguram A, DeLaughter DM, Conner DA, Marsiglia JDC, Kohli S, Chmatal L, Page DC, Zabaleta N, Vandenberghe L, Liu DR, Seidman JG, Seidman C. Efficient in vivo genome editing prevents hypertrophic cardiomyopathy in mice. Nat Med. 2023;29(2):412–21.
[216] Packer M. Cardioprotective effects of Sirtuin-1 and its downstream effectors: potential role in mediating the heart failure benefits of SGLT2 (Sodium-glucose cotransporter 2) inhibitors. Circ Heart Fail. 2020;13(9):e007197.
[217] Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, Rocca HPBrunner-La, Choi DJ, Chopra V, Chuquiure-Valenzuela E, Giannetti N, Gomez-Mesa JE, Janssens S, Januzzi JL, Gonzalez-Juanatey JR, Merkely B, Nicholls SJ, Perrone SV, Piña IL, Ponikowski P, Senni M, Sim D, Spinar J, Squire I, Taddei S, Tsutsui H, Verma S, Vinereanu D, Zhang J, Carson P, Lam CSP, Marx N, Zeller C, Sattar N, Jamal W, Schnaidt S, Schnee JM, Brueckmann M, Pocock SJ, Zannad F, Packer M. Empagliflozin in heart failure with a preserved ejection fraction. N Engl J Med. 2021;385(16):1451–61.
[218] Cowie MR, Fisher M. SGLT2 inhibitors: mechanisms of cardiovascular benefit beyond glycaemic control. Nat Rev Cardiol. 2020;17(12):761–72.
[219] Philippaert K, Kalyaanamoorthy S, Fatehi M, Long W, Soni S, Byrne NJ, Barr A, Singh J, Wong J, Palechuk T, Schneider C, Darwesh AM, Maayah ZH, Seubert JM, Barakat K, Dyck JRB, Light PE. Cardiac late Sodium Channel current is a molecular target for the Sodium/Glucose cotransporter 2 inhibitor empagliflozin. Circulation. 2021;143(22):2188–204.
[220] Hegyi B, Mira Hernandez J, Shen EY, Habibi NR, Bossuyt J, Bers DM. Empagliflozin reverses late Na(+) current enhancement and cardiomyocyte proarrhythmia in a translational murine model of heart failure with preserved ejection fraction. Circulation. 2022;145(13):1029–31.
[221] Mustroph J, Wagemann O, Lücht CM, Trum M, Hammer KP, Sag CM, Lebek S, Tarnowski D, Reinders J, Perbellini F, Terracciano C, Schmid C, Schopka S, Hilker M, Zausig Y, Pabel S, Sossalla ST, Schweda F, Maier LS, Wagner S. Empagliflozin reduces Ca/calmodulin-dependent kinase II activity in isolated ventricular cardiomyocytes. ESC Heart Failure. 2018;5(4):642–8.
[222] Baartscheer A, Schumacher CA, Wüst RCI, Fiolet JWT, Stienen GJM, Coronel R, Zuurbier CJ. Empagliflozin decreases myocardial cytoplasmic Na+ through inhibition of the cardiac Na+/H+ exchanger in rats and rabbits. Diabetologia. 2017;60(3):568–73.
[223] Uthman L, Baartscheer A, Bleijlevens B, Schumacher CA, Fiolet JWT, Koeman A, Jancev M, Hollmann MW, Weber NC, Coronel R, Zuurbier CJ. Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: inhibition of Na(+)/H(+) exchanger, lowering of cytosolic Na(+) and vasodilation. Diabetologia. 2018;61(3):722–6.
[224] Hundertmark MJ, Agbaje OF, Coleman R, George JT, Grempler R, Holman RR, Lamlum H, Lee J, Milton JE, Niessen HG, Rider O, Rodgers CT, Valkovič L, Wicks E, Mahmod M, Neubauer S. Design and rationale of the EMPA-VISION trial: investigating the metabolic effects of empagliflozin in patients with heart failure. ESC Heart Failure. 2021;8(4):2580–90.
[225] Chowdhury S, Jackson L, Byrne BJ, Bryant RM, Cade WT, Churchill TL, Buchanan J, Taylor C. Longitudinal observational study of cardiac outcome risk factor prediction in children, adolescents, and adults with Barth syndrome. Pediatr Cardiol. 2022;43:1251–63.
[226] Bertero E, Nickel A, Kohlhaas M, Hohl M, Sequeira V, Brune C, Schwemmlein J, Abeßer M, Schuh K, Kutschka I, Carlein C, Münker K, Atighetchi S, Müller A, Kazakov A, Kappl R, von der Malsburg K, van der Laan M, Schiuma AF, Böhm M, Laufs U, Hoth M, Rehling P, Kuhn M, Dudek J, von der Malsburg A, Prates Roma L, Maack C. Loss of mitochondrial Ca(2+) uniporter limits inotropic reserve and provides trigger and substrate for arrhythmias in Barth syndrome cardiomyopathy. Circulation. 2021;144(21):1694–713.
[227] Wang S, Li Y, Xu Y, Ma Q, Lin Z, Schlame M, Bezzerides VJ, Strathdee D, Pu WT. AAV gene therapy prevents and reverses heart failure in a murine knockout model of Barth syndrome. Circ Res. 2020;126(8):1024–39.
[228] Reid Thompson W, Hornby B, Manuel R, Bradley E, Laux J, Carr J, Vernon HJ. A phase 2/3 randomized clinical trial followed by an open-label extension to evaluate the effectiveness of elamipretide in Barth syndrome, a genetic disorder of mitochondrial cardiolipin metabolism. Genet Med. 2021;23(3):471–8.
[229] Thompson R, Jefferies J, Wang S, Pu WT, Takemoto C, Hornby B, Heyman A, Chin MT, Vernon HJ. Current and future treatment approaches for Barth syndrome. J Inherit Metab Dis. 2022;45(1):17–28.
[230] Han X, Yang J, Cheng H, Yang K, Abendschein DR, Gross RW. Shotgun lipidomics identifies cardiolipin depletion in diabetic myocardium linking altered substrate utilization with mitochondrial dysfunction. Biochemistry. 2005;44(50):16684–94.
[231] Han X, Yang J, Yang K, Zhao Z, Abendschein DR, Gross RW. Alterations in myocardial cardiolipin content and composition occur at the very earliest stages of diabetes: a shotgun lipidomics study. Biochemistry. 2007;46(21):6417–28.
[232] Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Federici A, Ruggiero FM. Decrease in mitochondrial complex I activity in ischemic/reperfused rat heart: involvement of reactive oxygen species and cardiolipin. Circ Res. 2004;94(1):53–9.
[233] Dolinsky VW, Cole LK, Sparagna GC, Hatch GM. Cardiac mitochondrial energy metabolism in heart failure: role of cardiolipin and sirtuins. Biochim Biophys Acta. 2016;1861(10):1544–54.
[234] Lioncino M, Monda E, Verrillo F, Moscarella E, Calcagni G, Drago F, Marino B, Digilio MC, Putotto C, Calabrò P, Russo MG, Roberts AE, Gelb BD, Tartaglia M, Limongelli G. Hypertrophic cardiomyopathy in RASopathies: diagnosis, clinical characteristics, prognostic implications, and management. Heart Fail Clin. 2022;18(1):19–29.
[235] Niemeyer CM. RAS diseases in children. Haematologica. 2014;99(11):1653–62.
[236] Nakamura T, Colbert M, Krenz M, Molkentin JD, Hahn HS, Dorn 2nd GW, Robbins J. Mediating ERK 1/2 signaling rescues congenital heart defects in a mouse model of Noonan syndrome. J Clin Invest. 2007;117(8):2123–32.
[237] Wu X, Simpson J, Hong JH, Kim KH, Thavarajah NK, Backx PH, Neel BG, Araki T. MEK-ERK pathway modulation ameliorates disease phenotypes in a mouse model of

Noonan syndrome associated with the raf 1(L613V) mutation. J Clin Invest. 2011;121 (3):1009–25.
[238] Hernández-Porras I, Fabbiano S, Schuhmacher AJ, Aicher A, Cañamero M, Cámara JA, Cussó L, Desco M, Heeschen C, Mulero F, Bustelo XR, Guerra C, Barbacid M. K-RasV14I recapitulates Noonan syndrome in mice. Proc Natl Acad Sci. 2014;111(46): 16395–400.
[239] Andelfinger G, Marquis C, Raboisson MJ, Théoret Y, Waldmüller S, Wiegand G, Gelb BD, Zenker M, Delrue MA, Hofbeck M. Hypertrophic cardiomyopathy in Noonan syndrome treated by MEK-inhibition. J Am Coll Cardiol. 2019;73(17):2237–9.
[240] Tomasovic A, Brand T, Schanbacher C, Kramer S, Hümmert MW, Godoy P, Schmidt-Heck W, Nordbeck P, Ludwig J, Homann S, Wiegering A, Shaykhutdinov T, Kratz C, Knüchel R, Müller-Hermelink HK, Rosenwald A, Frey N, Eichler J, Dobrev D, El-Armouche A, Hengstler JG, Müller OJ, Hinrichs K, Cuello F, Zernecke A, Lorenz K. Interference with ERK-dimerization at the nucleocytosolic interface targets pathological ERK1/2 signaling without cardiotoxic side-effects. Nat Commun. 2020;11(1):1733.
[241] Pieroni M, Moon JC, Arbustini E, Barriales-Villa R, Camporeale A, Vujkovac AC, Elliott PM, Hagege A, Kuusisto J, Linhart A, Nordbeck P, Olivotto I, Pietilä-Effati P, Namdar M. Cardiac involvement in fabry disease: JACC review topic of the week. J Am Coll Cardiol. 2021;77(7):922–36.
[242] Palecek T, Bultas J, Hajek M, Karetova D, Kuchynka P, Kautzner J, Elleder M, Linhart A. Association between cardiac energy metabolism and gain of left ventricular mass in fabry disease. Int J Cardiol. 2010;144(2):337–9.
[243] Machann W, Breunig F, Weidemann F, Sandstede J, Hahn D, Köstler H, Neubauer S, Wanner C, Beer M. Cardiac energy metabolism is disturbed in fabry disease and improves with enzyme replacement therapy using recombinant human galactosidase A. Eur J Heart Fail. 2011;13(3):278–83.