

# **OXIDATIVE STRESS IN CARDIAC HYPERTROPHY: FROM MOLECULAR MECHANISMS TO NOVEL THERAPEUTIC TARGETS**

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Running title: Oxidative stress and cardiac hypertrophy

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## **ABSTRACT**

When faced with increased workload the heart undergoes remodelling, where it increases its muscle mass in an attempt to preserve normal function. This is referred to as cardiac hypertrophy and if sustained, can lead to impaired contractile function. Experimental evidence supports oxidative stress as a critical inducer of both genetic and acquired forms of cardiac hypertrophy, a finding which is reinforced by elevated levels of circulating oxidative stress markers in patients with cardiac hypertrophy. These observations formed the basis for using antioxidants as a therapeutic means to attenuate cardiac hypertrophy and improve clinical outcomes. However, the use of antioxidant therapies in the clinical setting has been associated with inconsistent results, despite antioxidants having been shown to exert protection in several animal models of cardiac hypertrophy. This has forced us to reevaluate the mechanisms, both upstream and downstream of oxidative stress, where recent studies demonstrate that apart from conventional mediators of oxidative stress, metabolic influences as well as impairment in other cellular processes contribute to disease pathophysiology. Importantly, novel therapeutic interventions have been identified that can normalise such impairments, thereby counteracting oxidative stress with accompanying attenuation of cardiac hypertrophy and improved cardiac function. Here, we review the latest literature on these novel mechanisms and intervention strategies with the aim of better understanding the complexities of oxidative stress for more precise targeted therapeutic approaches to prevent cardiac hypertrophy.

## **KEYWORDS**

Oxidative stress; Reactive oxygen species; Cardiac hypertrophy; Antioxidants; Metabolism; Inflammation

## INTRODUCTION

Cardiac hypertrophy is a condition where the heart increases its muscle mass in response to intrinsic or external stress. Though considered adaptive at first to accommodate the demands of increased cardiac workload and biomechanical stress, sustained hypertrophy can lead to maladaptive remodelling with accompanying contractile dysfunction. Cardiac hypertrophy is usually associated with enlargement of the left ventricle (LV), although enlargement of the right ventricle (RV) is not uncommon [1]. Various aetiologies have been described for cardiac hypertrophy, including pressure overload (acquired) and mutations in sarcomeric and non-sarcomeric genes (inherited), and hence it is not surprising that cardiac hypertrophy is commonly observed in the setting of hypertension, hypertrophic cardiomyopathy (HCM), valvular disease and congenital heart disease [2].

With regard to the molecular pathogenesis, oxidative stress has been identified as a key mediator of cardiac hypertrophy across several aetiologies. In support of this, when five mouse models of cardiac hypertrophy with differing aetiology were compared, six cellular processes were found to be common, including oxidative stress, aging, contraction, developmental processes, cell differentiation, and cell proliferation [3]. More recently, OXY-SCORE, a global indicator of oxidative stress that combines individual plasma markers of oxidative damage and antioxidant capacity was able to differentiate between those with and without left ventricular hypertrophy (LVH), independent of other risk factors [4]. Similarly, high plasma protein carbonyl levels (a biomarker of oxidative protein modification) have been found to be an independent predictor of eccentric LVH in chronic haemodialysis patients [5]. Moreover, when assessing the association between oxidative stress biomarkers and cardiovascular risk factors and LVH in children with chronic kidney disease, hypertension and dyslipidaemia were found to correlate with lipid oxidation while oxidised low-density lipoprotein seemed to be an important marker correlating with LVH [6]. These studies highlight oxidative stress to be a critical pathophysiological feature in this setting cardiac hypertrophy.

Oxidative stress is considered the imbalance between reactive oxygen species (ROS) formation and cellular antioxidant capacity due to enhanced ROS generation and/or dysfunction of the antioxidant system. ROS can be generated from several cellular sources, including nicotinamide adenine dinucleotide phosphate oxidase (NOX), xanthine oxidase and the mitochondrial electron transport chain [7] and for a comprehensive review of the types of species generated we would like to direct the reader to the following excellent reviews [8, 9]. It is important to note that ROS production is a normal physiological process and is essential for redox balance, however, uncontrolled ROS overproduction has been found to induce protein and lipid peroxidation as well as DNA mutagenesis that can lead to irreversible cell damage or death [10]. In the setting of cardiac hypertrophy, oxidative stress has been found to activate ROS-sensitive pro-hypertrophic and remodelling signalling cascades [11] and consistently, treatment with antioxidants have been shown to attenuate the hypertrophic response as evidenced by reduction in cardiac remodelling and improvements in cardiac function in various animal models [12]. However, antioxidant

treatment in the clinical setting has yielded inconsistent results [13], forcing us to reevaluate the mechanisms by which oxidative stress exerts its detrimental effects.

Recently, several studies have attempted to identify novel upstream mediators of oxidative stress as well as downstream signalling pathways by which oxidative stress impacts cellular processes. Importantly, these studies have shown that apart from pressure overload and inherited forms of cardiomyopathies, oxidative stress can arise through mechanisms involving metabolic disturbances, mitochondrial dysfunction and inflammation amongst other dysregulated cellular processes, thereby revealing oxidative stress to be an extremely complex phenomenon that may explain as to why targeting of certain arms of oxidative stress alone may not be beneficial as patients with cardiac hypertrophy could present one of more of the above perturbations.

With the aim of understanding the complexities associated with oxidative stress, here we review the recent literature regarding oxidative stress-mediated cardiac hypertrophy and evaluate the mechanisms by which it is induced as well as discuss novel therapeutic approaches that may have the potential for attenuating cardiac hypertrophy and improving clinical outcomes.

## **MEDIATORS OF OXIDATIVE STRESS**

### ***ROS generating enzymes***

Nicotinamide adenine dinucleotide phosphate oxidase (NOX) proteins are reported to generate ROS in a highly regulated manner [14]. Several NOX isoforms have been identified and show tissue-specific distribution out of which NOX2 and NOX4 are the predominant isoforms expressed in the heart. Dysregulated expression of NOX proteins has been proposed to contribute to the development of cardiac hypertrophy and cardiac dysfunction. Consistently, elevated levels of NOX2 have been associated with worsening systolic function by a mechanism involving downregulation of PPAR $\alpha$  [15], an effect that could be reversed with PPAR $\alpha$  activator fenofibrate or NOX2 inhibitor VAS2870. In the same study, transcriptomic analysis of PPAR $\alpha$ <sup>-/-</sup> mice subjected to pressure overload revealed alterations in oxidative stress and inflammatory pathways that overlapped with wild-type mice (also subjected to pressure overload), while NOX2<sup>-/-</sup> mice subjected to pressure overload exhibited minimal changes in these pathways. While this reinforces that dysregulated NOX2 expression is a critical inducer of oxidative stress and remodelling, its relationship with PPAR $\alpha$  requires further investigation, although it can be speculated that downregulation of PPAR $\alpha$ -target genes (e.g. carnitine palmitoyltransferase I and medium-chain acyl-CoA dehydrogenase) may lead to reductions in mitochondrial membrane potential, increased cellular ROS and calcium levels with accompanying perturbations in mitochondrial activity and ATP generation [16].

NOX4 is expressed primarily in the mitochondria of cardiomyocytes and initial studies have suggested that elevated NOX4 levels exert detrimental effects as its up-regulation in response to hypertrophic stimuli and aging was found to induce oxidative stress, apoptosis and cardiac dysfunction. This was attributed in part to mitochondrial dysfunction due to ROS production and cysteine oxidation of mitochondrial proteins

[17, 18]. Angiotensin II (a hypertrophic stimulus) has also been shown to increase NOX4 levels which in turn induced nuclear export of HDAC4, a crucial suppressor of cardiac hypertrophy [19]. The dipeptidyl peptidase 4 inhibitor, teneligliptin, was able to attenuate cardiac hypertrophy and prevent increases in NOX4 mRNA, 4-hydroxynonenal and HDAC4 export by augmenting GLP-1 levels, while the GLP-1 receptor antagonist, exendin-3, abrogated teneligliptin-mediated protection, revealing the existence of a GLP-1/NOX4/HDAC4 axis.

In contrast to these findings, NOX4 has been shown to mediate protection against pressure overload by preserving myocardial capillary density [20], but not volume overload, where it promoted the latter by increasing Akt phosphorylation (as a result of NOX4-mediated SRC kinase-dependent inactivation of protein phosphatase 2A) and modulation of downstream proteins RPS6 and 4E-BP1 [21]. Moreover, NOX4<sup>-/-</sup> mice exhibited less eccentric LV remodelling and reduced cardiomyocyte enlargement that was attributed to reduction in phosphorylated Akt levels. Conversely, cardiomyocyte-targeted NOX4 overexpressing hearts were found to preferentially oxidise fatty acids for energy production, thereby improving myocardial bioenergetics in the setting of pressure overload [22]. Finally, NOX4 has been found to be down-regulated in the hearts of prenatally androgenised female offspring that exhibit cardiac hypertrophy in adulthood [23], highlighting the protective and diverse nature of this isoform.

Though NOX2 and NOX4 have been the main focus of oxidative stress, recent evidence suggest that other NOX proteins are also expressed in the heart and are mediators of cardiac hypertrophy. The calcium-regulated NOX5 isoform has been implicated in cardiac remodelling and contractile dysfunction that occurs in the setting of pressure overload [24]. In this study, overexpression of NOX5 was found to aggravate the hypertrophic response with transgenic NOX5 mice demonstrating greater magnitudes of hypertrophy, fibrosis and worsened contractile function. Treatment with either the ROS inhibitor N-acetylcysteine (NAC) or the L-type calcium channel blocker diltiazem was shown to mitigate the adverse effects mediated by NOX5 overexpression, thereby placing NOX5 as a key intermediate between ROS and calcium signalling networks. In other studies, lysyl oxidase (LOX), a copper-dependent amine oxidase that is reported to control matrix remodelling has also been found to aggravate the hypertrophic response, as mice overexpressing human LOX demonstrated heightened cardiac hypertrophy and cardiac dysfunction that was attributed to an enhanced fibrotic response as a result of excessive inflammation and ROS production which potentiated the activation of p38 MAPK while reducing AMP-activated protein kinase (AMPK) activation [25], suggesting new roles for LOX, apart from modulating collagen cross-linking.

In summary, based on several studies, dysregulated NOX expression has been found to be a key pathophysiological factor in the setting of cardiac hypertrophy (Figure 1). Further studies are needed to understand the interplay between the various NOX isoforms, their level of activation, and importantly, the downstream signalling pathways which they regulate. Future studies will need to take into consideration the differential responses of NOX proteins to varying types of hypertrophic stimuli

(pressure overload vs angiotensin II) [26]. Until then, the use of non-isoform selective NOX inhibitors for disease management should be viewed with caution.

### ***Metabolic disturbances***

Recently, several studies have shown diabetes and obesity to play detrimental roles in the progression of cardiac hypertrophy, thereby warranting a deeper understanding of the mechanisms involved (Figure 2). For instance, diabetic rats have been found to develop cardiac hypertrophy and fibrosis with increased myocardial levels of NOX2 and NOX4 [27]. Moreover, mice subjected to high-fat diet (HFD) were found to exhibit elevated NOX2 levels with accompanying increases in cardiomyocyte size, oxidative stress and aberrant redox signalling as well as increased Akt and Erk1/2 phosphorylation [28]. In this study, oxidative stress could be suppressed when heart homogenates were treated with the superoxide scavenger, Tiron, the flavohaemoprotein inhibitor diphenyleneiodonium and the NOX2 assembly inhibitor NOX2 ds-tat. Moreover, the above abnormalities were abrogated in NOX2-KO hearts, highlighting NOX2 as a key mediator of obesity-induced cardiac hypertrophy. Although the effect of NOX2-KO on cardiac function was not investigated in this study, but in another study improved cardiac systolic function has been observed in NOX2<sup>-/-</sup> mice in the setting of pressure overload [15]. It therefore appears that dysregulated NOX2 expression is a common determinant of both obesity- and pressure-induced cardiac hypertrophy. In other studies, oxidative stress induced by HFD was found to activate a BCL10/CARD9/p38 MAPK axis that could be suppressed by zinc-mediated activation of metallothionein [29], revealing the existence of signalling intermediates between oxidative stress and conventional inducers of cardiac hypertrophy.

Considering the detrimental effects mediated by HFD, it is not unreasonable to speculate that lipid metabolism regulatory pathways may contribute to disease progression or prevention. For instance, knockdown of CTRP9, a gene involved in the regulation of lipid metabolism was found to aggravate cardiac hypertrophy, fibrosis, endoplasmic reticulum stress-initiated apoptosis and oxidative stress in the setting of obesity-induced cardiac hypertrophy, effects which were attributed to decreased AMPK phosphorylation and increased mTOR phosphorylation [30]. Moreover, in the setting of palmitate-induced lipotoxicity, treatment with CTRP9 protein was shown to increase LKB1 phosphorylation which in turn promoted its cytoplasmic localisation that is critical for AMPK activation, highlighting anti-lipotoxic properties likely mediated through a LKB1/AMPK axis. In other studies, palmitate treatment has been found to induce cristae remodelling, mitochondrial fission and ROS generation [31], where mechanistic studies linked these phenomena to decreased AMPK expression which in turn inhibits mitofilin interaction with both SAM50 and CHCHD3, two core components of the MICOS complex. Interestingly, the major neurotransmitter acetylcholine was shown to alleviate cardiomyocyte hypertrophy by improving cristae remodelling and mitochondrial function which was likely mediated through increased mitofilin expression and AMPK activation.

Lipid kinases such as PIKfyve are reported to be key regulators of cardiometabolic status and mitochondrial integrity as it has been found to induce

mitochondrial ROS production and apoptosis [32]. Inhibition of PIKfyve through the use of a selective inhibitor STA was shown to suppress oxidative stress, apoptosis and mitochondrial damage which resulted in attenuated cardiac hypertrophy and improved cardiac function through a SIRT3-dependent pathway. Consistently, SIRT3 activation has been found to be protective against mitochondrial and energy metabolic dysfunction mediated by RIP140, a deleterious regulator of cardiac mitochondrial function and energy metabolic homeostasis [33]. Moreover, other lipid regulatory proteins like perilipin 5 (PLIN5) which is involved in the metabolism of lipid droplets and which is highly expressed in oxidative tissue such as in the heart have also been investigated. In the setting of pressure overload, mice deficient for PLIN5 were found to exhibit aggravated cardiac hypertrophy and dysfunction with accompanying reductions in cardiac lipid accumulation, increased mitochondrial biogenesis and fatty acid oxidation as well as heightened oxidative stress [34]. These observations support a role for PLIN5 in the protection of myocardial triglyceride stores, preservation of fatty acid oxidation, and prevention of oxidative stress.

Apart from excess fatty acids, hyperglycaemic conditions have also been shown to induce cardiomyocyte hypertrophy as incubation of H9c2 cells in high glucose concentrations resulted in increased cell size and increased cellular calcium and hypertrophic signals in addition to excess ROS production [35]. Interestingly, the PPAR $\delta$  agonist, GW0742, was shown to mitigate high glucose-induced cardiomyocyte hypertrophy by decreasing cellular calcium which in turn reduced ROS production and subsequent hypertrophic signalling. With regard to the mechanism, hyperglycaemic conditions were found to reduce thioredoxin 2 levels (a mitochondrial antioxidant that along with thioredoxin reductase 2 and peroxiredoxin 3 protects against oxidative stress) in both H9c2 cells and in myocardium of diabetic rats [36], highlighting a direct effect of hyperglycaemia on redox balance.

Although metabolic disturbances have been found to be associated with adverse cardiac remodelling it is unclear as to whether the accompanying oxidative stress is the primary cause of disease manifestation or secondary to other pathophysiological events, although recent evidence suggests the former. When cardiac metabolism, function and structure were evaluated in a time course study in spontaneously hypertensive rats, chronic pressure overload was found to induce increases in myocardial glucose uptake and oxidation as well as elevate oxidative stress and inflammation and induce metabolite abnormalities that coincide with, or precede cardiac dysfunction while LVH developed later on [37]. While it remains to be determined as to how these early metabolic changes give rise to LVH, the activation of mTOR has been proposed as a contributing factor. Similarly, profound myocardial metabolic changes have been found to occur prior to the progression of LVH and cardiac dysfunction in spontaneously hypertensive rats [38].

In light of these observations, the modulation of metabolism has gained much interest as a potential therapeutic approach for reversing adverse events associated with cardiac hypertrophy. For instance, the glucose lowering agent metformin was shown to mitigate early myocardial metabolic changes by normalising glucose uptake rates and reducing circulating free fatty acids (fatty acyl carnitines) in addition to

exerting positive effects on LV mass and LV wall thickness which resulted in improved cardiac function [38]. The beneficial effects of metformin have been attributed to the normalising of AMPK and mTOR signalling pathways as well as enhanced fatty acid oxidation and reductions in oxidative stress. Moreover, in a clinical study that evaluated the ability of metformin to reduce LVH in patients with coronary artery disease (with insulin resistance but without type 2 diabetes), metformin treatment was reported to remarkably reduce LV mass index, LV mass, systolic blood pressure, body weight and oxidative stress [39].

In other studies, the rate-limiting enzyme of fatty acid  $\beta$ -oxidation, short-chain acyl-CoA dehydrogenase (SCAD) is reported to negatively regulate cardiac hypertrophy and fibrosis [40]. Consistently, by increasing expression and enzyme activity of SCAD, flavin adenine dinucleotide treatment was shown to inhibit cardiomyocyte hypertrophy and cardiac fibroblast proliferation [41] that was attributed to increased fatty acid oxidation and decreased ROS production. Similarly, allylmethylsulfide, a novel sulphur metabolite of garlic was shown to attenuate cardiac hypertrophy and fibrosis by reducing oxidative stress (via reducing lipid peroxidation and improving exogenous antioxidant activity) and apoptosis in addition to stabilising extra cellular matrix components [42], likely mediated through  $\text{Na}^+/\text{K}^+$ -ATPase [43].

Finally, caloric restriction itself has been found to protect against adverse cardiac remodelling in diabetic and obese mouse models by normalising iron homeostasis which subsequently resulted in decreased oxidative stress and inflammation [44]. Moreover, caloric restriction was shown to attenuate cardiac hypertrophy by improving intracellular redox balance through a mechanism involving mitoKATP activation [45]. Although the precise anti-hypertrophic mechanism mediated by caloric restriction is undetermined, it could be speculated that caloric restriction reduces oxidative stress and increases cellular antioxidant capacity. Conversely, while chronic exercise was shown to attenuate cardiac hypertrophy and fibrosis, it was unable to restore the nitroso-redox imbalance imposed by diabetes [27].

In summary, the above findings highlight as to how metabolic disturbances could induce a hypertrophic response through mechanisms involving oxidative stress. Further studies are needed to assess as to whether oxidative stress impacts metabolic regulators with subsequent perturbations in substrate preference and bioenergetic impairment which is known to occur in the setting of cardiac hypertrophy.

### ***Mitochondrial dysfunction***

Mitochondria are the powerhouse of the cell and apart from generating ATP, these organelles play an important role in maintaining cellular redox balance. As a consequence of ATP production, the electron transport chain complexes are a major source of ROS generation and while this is tightly regulated by antioxidant defence systems, an imbalance between the production of free radicals and the ability to detoxify these species could result in oxidative stress (Figure 3). Unsurprisingly, disturbances in mitochondrial or antioxidant proteins have been associated with cardiac hypertrophy. In patients with HCM, mitochondrial complex I has been found to be upregulated with elevated ATP levels and increased antioxidant enzymes [46],

suggesting hyperactivity of complex I which was proposed to contribute to elevated ROS levels. ROS can also effect ATP levels as evidenced by a study which compared differential status of energy metabolism between myocardial infarction and pathological hypertrophy, where ATP levels were found to be significantly impaired in the former, while being preserved in the latter [47]. This phenomenon was attributed to a PGC1 $\alpha$ /ERR $\alpha$  axis that remained active during myocardial infarction, but not during cardiac hypertrophy. Mechanistic studies indicated ROS to negatively regulate NF- $\kappa$ B by oxidation of cysteine residues on its DNA binding element which allowed for the preservation of the PGC1 $\alpha$ /ERR $\alpha$  axis during myocardial infarction which in turn inhibited pyruvate dehydrogenase via activation of PDK4. Considering that ROS levels are also elevated during cardiac hypertrophy, further work is needed to determine why NF- $\kappa$ B was not deactivated (with subsequent activation of PGC1 $\alpha$ /ERR $\alpha$  signalling) in this setting.

Abnormal mitochondrial ion homeostasis has also been associated with cardiac hypertrophy. For instance, high-salt diet was found to increase cardiac mitochondrial TRPC3 expression which resulted in enhanced mitochondrial calcium uptake and ROS production [39]. This was accompanied by cardiac hypertrophy and mitochondrial dysfunction as evidenced by decreased ATP production and mitochondrial complex I and II enzyme activity. Moreover, as TRPC3 depletion was shown to attenuate cardiac hypertrophy and improve mitochondrial function, the importance of regulated ion uptake was highlighted in this study. Mitochondrial dysfunction as a result of iron overload has also been investigated in the setting of apelin-13-induced cardiac hypertrophy, where apelin-13 (an endogenous ligand for the G protein-coupled apelin receptor) was found to promote total cellular and mitochondrial iron production with accompanying increases in mitochondrial ROS [48]. Mechanistically, this phenomenon was attributed to increases in mitochondrial iron transporting protein SFXN1 and a cargo receptor for ferritinophagy, NCOA4 suggesting that the endogenous ferritinophagy pathway may be a critical molecular switch in the development of cardiac hypertrophy.

Perturbations in mtDNA has been shown to have severe consequences as accumulation of mtDNA damage with age has been found to induce cardiac hypertrophy and dilation with accompanying impairments in systolic and diastolic function partly mediate by mitochondrial oxidative stress [49]. Endonuclease G (ENDOG) which encodes for a mitochondrial nuclease has been identified as a determinant of cardiac hypertrophy, as its deficiency was found to induce ROS production and cell enlargement by altering the Akt/GSK3 $\beta$  and class-II HDAC signalling cascades [50]. Paradoxically, no changes were detected in the expression and activity of respiratory chain complexes nor in ATP content despite reductions in mtDNA as a consequence of ENDOG deficiency. Interestingly, treatment with humanin, a micropeptide coded by mtDNA was able to restore ROS levels and normalise cell size in ENDOG-deficient cardiomyocytes suggesting interplay between mtDNA content and cell growth.

The role of MTG1 that regulates mitochondrial ribosome assembly and translation has been investigated in cardiac hypertrophy [51]. While MTG1

overexpression was found to confer protection, MTG1 deficiency significantly exacerbated the hypertrophic phenotype. Mechanistic studies revealed MTG1 to preserve mitochondrial respiratory chain complex activity which in turn reduced ROS production in addition to downregulation of TAK1, p38 MAPK and Jnk1/2 stress signalling pathways in the setting of pressure overload. Considering that MTG1 was found to be elevated in the hearts of patients with dilated cardiomyopathy and in mice subjected to pressure overload, further investigation is needed to determine as to why there was lack of protection in these settings. In other studies, TIM50 was found to be down-regulated in hypertrophied hearts and was associated with an aggravated hypertrophic response characterised by increased cardiomyocyte size and fibrosis [52]. While TIM50 deficiency was found to signal through an ASK1/Jnk/p38 MAPK axis, the ability for the antioxidant NAC to reverse the aggravated phenotype would suggest TIM50 deficiency to be associated with oxidative stress. This was supported by findings that revealed TIM50 to regulate ROS generation and activities of superoxide dismutase and catalase as well as complexes I, II and IV of the electron transport chain. Finally, uncoupling between gap junction proteins and mitochondria function has also been observed in cardiac hypertrophy as evidenced by the decreased expression of connexin 43 and GJA1-20 in spontaneously hypertensive rats [53]. Although overexpression of GJA1-20 was shown to increase mitochondrial membrane potential and oxygen consumption rates while reducing cell size and attenuating ROS levels, these improvements observed on mitochondria function are likely to be secondary to the chaperoning of connexin 43, which was shown to be the primary function of GJA1-20.

In an attempt to protect mitochondria from oxidative damage, attention has been focussed on the development of antioxidants that can penetrate the mitochondria. In this regard, the mitochondria-targeted antioxidant MitoQ has been shown to protect against the development of cardiac hypertrophy likely mediated by improved endothelial function as a result of increased nitric oxide bioavailability [54]. Similarly, the combination of MitoQ10 and low-dose losartan has been shown to provide additive protection by attenuating the development of hypertension and reducing cardiac hypertrophy [55]. Alternatively, targeting of mitochondrial dynamics could also be considered as a potential therapeutic strategy as administration of Drp1 inhibitor mdivi-1 in the setting of hypertension was shown to attenuate LVH and reduce fibrosis in addition to decreasing ROS production which subsequently suppressed calcineurin and CaMKII expression [56].

In summary, these studies demonstrate that in addition to ATP production, mitochondria play a central role in preventing ROS accumulation. The close association between oxidative stress and perturbations in mtDNA, ion homeostasis, ribosome assembly, protein translocation as well as dynamics suggests mitochondrial dysfunction to be a critical mediator of oxidative stress-induced cardiac hypertrophy, thereby warranting a deeper understanding of the pathways involved.

## ***Inflammation***

Inflammation is a complex biological response to cellular or tissue damage and is associated with myocardial fibrosis, diastolic dysfunction and cardiac hypertrophy [57]. Whether inflammation induces oxidative stress in the setting of cardiac hypertrophy is less understood. Toll-like receptor 4 (TLR4) signalling is a critical link between oxidative stress, inflammation and cardiovascular disease [58]. In support, in a rat model of pre-term birth that was exposed to high levels of oxygen, an increase in TLR4 signalling was found to induce ROS production, inflammation and CD68<sup>+</sup> macrophage infiltration which resulted in cardiac hypertrophy, fibrosis and cardiac dysfunction, all of which were prevented upon treatment with a TLR4 antagonist [59]. Moreover, TLR4 expression has also been found to be markedly increased with accompanying mitochondrial dysfunction and disturbed cellular antioxidant flux during isoproterenol-induced cardiac hypertrophy [60]. In this study, while these perturbations were shown to be prevented in the presence of a TLR4 receptor inhibitor RS-LPS, treatment with the TLR4 agonist LPS aggravated oxidative stress and accelerated disease progression, thereby highlighting inflammation as a crucial mediator of cardiac hypertrophy through induction of oxidative stress.

Nrf2 is a transcription factor that is reported to play a critical role in preventing oxidative stress and cardiac hypertrophy as its deficiency has been found to aggravate cardiac hypertrophy, fibrosis, oxidative stress, inflammation and cardiac dysfunction through an IL-6/STAT3 axis [61]. In an attempt to preserve Nrf2 signalling, overexpression of the antioxidant peroxiredoxin 1 exerted protection through a Nrf2/HO-1 axis which led to decreased inflammation and oxidative stress [62]. Interestingly, though peroxiredoxin 1 was inherently upregulated in the setting of pressure overload, these levels were not sufficient to prevent disease onset, allowing for the speculation that other signalling intermediates could be present in this cascade. Moreover, inflammation has also been found to signal through regulators of DNA as the transcriptional and epigenetic regulator bromodomain-containing protein 4 (BRD4) was found to be upregulated in cardiomyocytes following exposure to angiotensin II. This was attributed to increased ROS generation as both NAC treatment and BRD4 suppression were able to limit cell enlargement, repress TGF $\beta$ 1/SMAD signalling pathways as well as alleviate inflammation and oxidative stress through inhibition of NF- $\kappa$ B signalling and improved Nrf2/HO-1 signalling, respectively [63]. In the same study, BRD4 deficient mice were reported to exhibit protection against pressure overload as evidenced by reductions in cardiac hypertrophy, fibrosis, inflammation, oxidative stress and cardiac dysfunction.

Adipokines are inflammatory mediators that are also associated with cardiac hypertrophy as cardiomyocyte-specific adipokine, CTRP3, has been found to be upregulated in murine hypertrophic hearts and failing human hearts [64]. The increase in CTRP3 was found to be induced by ROS during the hypertrophic response and while overexpression of CTRP3 worsened cardiac hypertrophy and function, CTRP3 deficiency alleviated the hypertrophic phenotype. Mechanistic studies revealed CTRP3 to signal through a TAK1/Jnk axis that was initiated by PKA. Moreover, secretory proteins have also been investigated in an attempt to identify their role in cardiac hypertrophy. FNDC5 plays an important role during muscle contraction in that

it undergoes cleavage to release the myokine irisin into the bloodstream which in turn promotes mitochondrial biogenesis and increases metabolic rate in cardiomyocytes [58]. In support, FNDC5 deficiency in the setting of HFD aggravated cardiac hypertrophy and increased inflammatory cytokine expression and oxidative stress that was attributed to activation of the JAK2/STAT3 pathway [65]. Although it remains to be determined as to how FNDC5 modulates the JAK2/STAT3 signalling axis, this study highlights the importance of myokines in maintaining cardiac homeostasis. Finally, treatment with FGF21 (a hepatokine) was found to attenuate cardiac hypertrophy, fibrosis, apoptosis and cardiac dysfunction that was likely mediated by an increase in the deacetylase activity of SIRT1, which promoted its interaction with LKB1 and FOXO1 [66]. While deacetylation of LKB1 resulted in activation of AMPK, deacetylation of FOXO1 led to altered transcriptional activity which resulted in increased expression of catalase and MnSOD and decreased expression of pro-apoptotic protein BIM. These findings support a critical role of adipokines, myokines and hepatokines in regulating inflammatory responses in the setting of cardiac hypertrophy.

In other studies, molecules associated with the innate immune system like C1QTNFs have been reported to display differential effects on metabolic homeostasis which could influence the cardiovascular system [67]. Consistently, C1QTNF1 has been found to be elevated in response to ROS whereas its deficiency led to accelerated cardiac hypertrophy and remodelling with increased inflammation and oxidative stress and worsened cardiac function [68]. Introduction of the recombinant human globular domain of C1QTNF1 mitigated the hypertrophic response through AMPK $\alpha$  activation which in turn suppressed mTOR and P70S6K phosphorylation with mechanistic studies revealing the existence of a cAMP/PKA/LKB1 axis that was a critical prerequisite for C1QTNF1-mediated AMPK $\alpha$  activation.

Other cell types have also been reported to promote an inflammatory response in the setting of cardiac hypertrophy. For instance, endothelial cells have been found to secrete endothelial microparticles (EMP) containing mitochondria and if these mitochondria were dysfunctional (excessive ROS production), EMPs were then able to induce expression of proinflammatory mediators in endothelial cells which in turn increased monocyte adhesion [69]. Mechanistically, MK2 was found to regulate EMP generation during inflammation by increasing E-selectin expression and cytoskeletal rearrangement through ROCK2. In the same study, MK2 inhibition was shown to attenuate cardiac hypertrophy and fibrosis as well as improve cardiac function, highlighting as to how paracrine mediators such as EMP could influence disease pathophysiology. Consistently, when investigating the relationship between plasma xanthine oxidase (a product of endothelial cells) and LVH in patients with resistant hypertension (RHTN), a two-fold increase in xanthine oxidase was observed in RHTN patients as compared to normotensive persons which was positively correlated with LV mass, LV diastolic function and 24-hour urinary sodium [70], highlighting an association between endothelium-derived oxidative stress and excess dietary salt in LVH pathophysiology.

In summary, based on these observations it would seem that not only does inflammation induce oxidative stress but the reverse is also true, suggestive of a feedback loop between these two physiological processes. Moreover, though inflammation is a well-documented occurrence in the setting of cardiac hypertrophy, not all mediators of inflammation are detrimental and hence, more studies are needed to delineate the relationship between pro- and anti-inflammatory pathways with regard to oxidative stress, as suppression of the wrong cascade could have deleterious outcomes (Figure 4).

### **Autophagy**

Cardiomyocyte growth is a key pathophysiological feature of cardiac hypertrophy, necessitating the need for understanding its regulation. In support, CDC20, an anaphase-promoting complex activator essential for cell division has been found to be up-regulated in the hypertrophied heart [71]. While knockdown of CDC20 attenuated cardiac hypertrophy, ectopic expression of CDC20 aggravated the hypertrophic response by directly targeting and promoting ubiquitination and degradation of the critical autophagy regulator LC3, thereby impairing autophagy and promoting cardiac hypertrophy. This study highlights a critical link between cell division proteins, the autophagy machinery and the hypertrophic response.

Apart from acquired LVH and HCM, Fabry disease is a rare genetic disorder caused by defects in the alpha-galactosidase (GLA) enzyme which results in globotriaosylceramide (Gb3) accumulation. Although considered a multi-system disorder, LVH is a key pathophysiological feature being reported in up to 50% of males and one-third of females [72]. Consistently, when the GLA gene was knocked out in human embryonic stem cells (hESCs), the differentiated cardiomyocytes were found to exhibit cellular hypertrophy with accompanying accumulation of Gb3 and decreases in the Rab GTPases involved in exocytotic vesicle release [73]. This resulted in impaired autophagy flux and protein turnover which in turn induced ROS production and apoptosis, however, further work is needed to determine the interplay between exosome biogenesis and autophagy.

Since impaired autophagy is known to destabilise proteostasis and elevate intracellular oxidative stress during cardiac remodelling, targeting this pathway has been proposed as a viable strategy for mitigating the hypertrophic phenotype. In support, oridonin, the major active ingredient of the traditional Chinese medicinal herb *Rabdosia rubescens* was shown to attenuate cardiac hypertrophy and preserve heart function in the setting of pressure overload [74]. While the beneficial effects of oridonin were attributed to increased antioxidant activities and suppressed oxidative injury (which were a result of intracellular protein accumulation), mechanistically this was linked to enhanced myocardial autophagy mediated by increased levels of cytosolic p21 which reduced Akt but increased AMPK phosphorylation. While enhancing autophagy appears to be an attractive strategy, caution is advised as excessive autophagy has been reported to be maladaptive. In support, hypertrophic stimuli were found to induce autophagy through a mechanism involving NOX2. Interestingly, stachydrine, a major constituent of *Leonurus heterophyllus* Sweet was shown to inhibit

NOX2 activity which led to reductions in ROS and subsequent attenuation of cardiac hypertrophy and cardiac dysfunction as well as decreased excessive autophagy [75]. Moreover, apelin-13 was found to promote cellular hypertrophy through an endoplasmic reticulum stress-autophagy pathway as evidenced by increases in ROS production, NOX4 expression, endoplasmic reticulum stress markers (BiP and CHOP) and autophagy markers (LC3-II/I and beclin-1) [76], suggesting interplay between ROS, endoplasmic reticulum stress and autophagy.

In summary, while studies suggest autophagy to be involved in both promoting and preventing cardiac hypertrophy (Figure 5), the occurrence of reticulophagy (a selective form of autophagy of the endoplasmic reticulum) was found to occur in the setting of apelin-13 induced cardiomyocyte hypertrophy and further investigation is needed to determine as to how such specificity arises [76].

### ***Protein quality control***

Protein quality control is a cellular phenomenon through which aberrant proteins are eliminated and hence, maintaining functional protein homeostasis is important as disruptions in these pathways could have detrimental effects on the cell. In the setting of cardiac hypertrophy TRAF6, a ubiquitin E3 ligase was found to be elevated in human and murine hypertrophied hearts [77]. Mechanistically, TRAF6 was found to be regulated by ROS generated during the hypertrophic progression which triggered its auto-ubiquitination that led to the formation of a TRAF6-TAK1 interaction that was indispensable for cardiac remodelling. Moreover, REG $\gamma$ , a member of the 11S proteasome activator is reported to bind and activate the 20S proteasome to promote the degradation of several proteins [78]. In the setting of pressure overload, REG $\gamma$  was found to be up-regulated and associated with increased decay of PP2A $\alpha$ , which resulted in increased phosphorylation and nuclear export of FOXO3a which subsequently led to a decline in MnSOD and ROS accumulation [79]. In the same study, ectopic expression of PP2A $\alpha$  or MnSOD was shown to mitigate REG $\gamma$ -mediated ROS accumulation and treatment with a MnSOD mimetic, MnTBAP was found to prevent ROS accumulation and cardiac hypertrophy highlighting REG $\gamma$ /PP2A $\alpha$ -FOXO3a/MnSOD as an important signalling pathway (Figure 5).

In other studies, continuous overexpression of the small heat shock protein Hsp22 in mice has been found to increase ROS production which in turn induced cardiac hypertrophy, senescence (as evidenced by increased p16 and p19 levels, percentage of  $\beta$ -galactosidase positive cells and telomerase activity) and a reduction in lifespan [80]. In this study, the 3 major cellular sources of ROS (NADPH oxidase, xanthine oxidase and complex I) were found to have elevated activity that could be abolished with the antioxidant tempol which in turn attenuated cardiac hypertrophy, prevented senescence and extended the life span likely through modulation of Akt phosphorylation. Although it remains to be determined as to how Hsp22 enhanced the activity of ROS producing proteins, it can be speculated that this could be due to their stabilisation, as Hsp22 is reported to have chaperoning properties [81].

In summary, studies have shown dysregulated protein quality control to be a mediator of oxidative stress and cardiac hypertrophy through heightened proteasomal

activity that results in the perturbation of protective pathways and in the probable stabilisation of ROS producing enzymes.

### ***miRNAs***

miRNAs are small non-coding RNA molecules that are involved in the post-transcriptional regulation of genes. Several miRNAs have been found to commonly contribute to cardiac hypertrophy. For instance, miR-29 has been implicated in HCM where its silencing has been associated with increased cardiac fibrosis [82]. With regard to its regulation, endothelin-1 has been proposed as an upstream regulator of miR-29a as treatment of cardiomyocytes with endothelin-1 was found to increase ROS and suppress miR-29 with corresponding increase in TGF $\beta$  expression, which in turn stimulated collagen expression in fibroblasts. In the same study, it was found that suppression of miR-29 was only observed in the R92W-TnT mouse model and not in the R403Q-MyHC model nor in human myomectomy samples, where the latter surprisingly demonstrated activation of anti-hypertrophic/anti-fibrotic signalling pathways. It can be speculated that while differences between the two models may be due to their differential redox status, the activation of anti-hypertrophic/anti-fibrotic signalling pathways in human samples may be a result of compensatory mechanisms.

Conversely, the levels of miR-200c has been found to be increased during cardiac hypertrophy and has been shown to be a direct target of MLCK and hence, an increase in miR-200c induced a decrease in phosphorylated MLC2 [83]. Interestingly, down-regulation of miR-200c using a specific inhibitor was shown to attenuate cardiomyocyte hypertrophy and reduce apoptosis as well as ROS accumulation, effects that were lost upon MLCK inhibition, thereby highlighting a direct link between miRNAs, sarcomere function and ROS.

In summary, though dysregulation of miRNAs has been found to occur during cardiac hypertrophy (Figure 5) it is unclear as to whether they mediate disease pathology through mechanisms involving oxidative stress, although miRNAs have been found to regulate mitochondrial proteins in response to hypertrophic stimuli, as miR-28 was found to target the 3' untranslated region of VDAC1 [16], while miR-106a was found to target Mfn2 resulting in cristae defects, depolarization of mitochondrial membrane and increased ROS production [84], allowing for the speculate of an association between miRNAs, mitochondrial protein and oxidative stress.

### ***Ion channels***

Cardiac ion channel complexes form the basis for excitation contraction coupling including calcium-induced calcium release and mechanical contraction and while ion channel dysregulation has been reported in cardiac hypertrophy [85], whether this is also associated with oxidative stress is less clear. In spontaneously hypertensive rats, the existence of an EGFR/NHE1 axis has been proposed as EGFR depletion led to reduction in NHE1 activity with accompanying attenuation of cardiac hypertrophy and decreased fractional shortening [86]. Although this signalling cascade requires further investigation, it can be speculated that NHE1 activation is ROS-dependent as EGFR depletion resulted in reduced NHE1 activity, albeit with no change in protein levels but

with accompanying reductions in ROS and lipid peroxidation. In other studies, the expression and activity of NKA was found to be decreased in rodent cardiomyocytes exposed to angiotensin II [87]. Interestingly, treatment with an antibody against the 4<sup>th</sup> extracellular region of NKA (DR-Ab) was shown to be protective as this led to stabilisation of NKA on the plasma membrane and restored its activity with accompanying reductions in ROS through NOX inhibition. Although mechanistic studies linked these beneficial effects of DR-Ab to the activation of an AMPK/SIRT3/PPAR $\gamma$  axis, further studies are needed to validate the relationship between ion channel expression/activity and oxidative stress (Figure 6).

Recently, enhancement of the plasma membrane small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels in hypertrophied hearts were shown to mediate protection against arrhythmia by attenuating mitochondrial ROS and subsequently decreasing oxidation of reactive cysteine residues on ryanodine [88]. An association between impaired calcium handling and increased ROS production has also been observed in a hESC model of inherited hypertrophic cardiomyopathy caused by mutations in the muscle LIM protein (MLP), a key regulator of striated muscle function [89]. MLP deficiency was found to induce a hypertrophic phenotype in hESC-derived cardiomyocytes with accompanying mitochondrial damage, increased ROS production and impaired calcium handling. Interestingly, the calcium channel blocker verapamil was shown to prevent the development of HCM suggesting elevated intracellular calcium concentration to be a central mechanism for MLP-deficiency induced cardiac hypertrophy, although assessment of verapamil in reducing ROS levels was not investigated.

In summary, oxidative stress has been found to have a profound effect on cardiac ion channels. Considering the criticality of ion channels in regulating excitation contraction coupling, an extensive understanding of oxidative stress-induced modifications could help to identify potential therapies that could prevent incidences of arrhythmia, which is a common occurrence in patients with cardiac hypertrophy.

## **THERAPEUTIC STRATEGIES FOR ATTENUATING OXIDATIVE STRESS**

### ***Antioxidants***

Considering that oxidative stress is a result of impaired antioxidant activities, several studies have focussed on augmenting these signalling cascades through direct or indirect intervention. In this regard, several naturally occurring compounds have been shown to alleviate the hypertrophic phenotype through suppression of oxidative stress. For instance, the pulp powder extracted from *Musa balbisiana* (banana) [90], the lowbush blueberry extract (*Vaccinium angustifolium*) [91], the aqueous extract of the *Terminalia arjuna* bark [92] and pomegranate juice [93] have been associated with reduction in heart weight, fibrosis, inflammation and oxidative stress. In addition to suppressing cardiac hypertrophy, these compounds have been reported to exert beneficial effects across several pathologies suggestive of pleiotropic function. Moreover, while the proposed mode of action of these compounds is through suppression of oxidative stress, likely mediated by several phenolic antioxidants, the exact composition and the signalling pathways through which they signal are less

defined, although quercetin, kaempferol, boeravinone B and caffeic acid have been identified in the ethanolic extract of *Boerhavia diffusa* [94].

Signalling cascades that regulate oxidative stress and/or antioxidant activity have however been identified in other studies. Nobiletin, a polymethoxy flavonoid was shown to attenuate cardiac hypertrophy by blunting the increased expression of NOX2 and NOX4 as well as alleviating endoplasmic reticulum stress and reducing cardiomyocyte apoptosis in the setting of pressure-overload [95]. In other studies, dihydromyricetin, a flavonoid in vine tea, has been reported to exert protection by reducing ROS and lipid peroxidation while increasing antioxidant expression and activity which was attributed to enhanced SIRT3 signalling [96]. However, not all members of the SIRT family are protective, and caution should be observed when targeting these proteins as SIRT4 overexpressing mice were found to exhibit aggravated hypertrophy, fibrosis and cardiac dysfunction in response to angiotensin II. This was attributed to less binding of MnSOD to SIRT3 which resulted in increased MnSOD acetylation and reduced activity, thereby inducing ROS accumulation [97]. Importantly, a mimetic of superoxide dismutase, manganese 5, 10, 15, 20-tetrakis-(4-benzoic acid) porphyrin was shown to exert beneficial effects through inhibition of ROS.

While these compounds exert protection by augmenting antioxidant pathways and suppressing oxidative stress, their pleiotropic nature has been found to influence off-target signalling, albeit with beneficial effects. The natural flavonoid, delphinidin for instance has been shown to attenuate hypertrophic growth, reduce fibrosis and improve cardiac function which was attributed to its ability to reduce ROS accumulation by activating AMPK with subsequent inhibition of Rac1 (NOX activator) activity and p47<sup>phox</sup> (a NOX subunit) expression [98]. Interestingly, delphinidin also abrogated oxidative stress-mediated increases in Erk1/2, p38 MAPK and Jnk1/2 phosphorylation, suggesting modulation of an AMPK/NOX/MAPK axis. Similarly, fisetin, a small molecular flavonoid was found to attenuate cardiac hypertrophy and improve cardiac function by decreasing ROS levels and up-regulating antioxidant genes including catalase, superoxide dismutase and HO-1 in addition to suppressing pro-hypertrophic signalling pathways such as MAPK and mTOR [99]. In other studies, astragaloside IV, a bioactive saponin isolated from the dried plant roots of the genus *Astragalus*, has been shown to alleviate the hypertrophic phenotype by increasing Nrf2 and HO-1 levels and suppressing ROS [100], astragaloside IV was also found to function through elevating SIKE (a negative regulator of the interferon pathway) which in turn suppressed the TBK1/PI3K/Akt axis [101]. Moreover, rutaecarpine an alkaloid isolated from *Evodia rutaecarpa*, the most popular and multi-purpose herb traditionally used in China for treatment of several symptoms, was shown to attenuate cardiac hypertrophy through suppression of both a NOX4/ROS/ADAM17 axis and the pro-hypertrophic Erk1/2 pathway [102]. Additionally, ginsenoside Rd, one of the main active ingredients in *Panax ginseng*, was also reported to improve cardiac function and attenuate cardiomyocyte hypertrophy, fibrosis, inflammation and oxidative stress with accompanying reductions in Akt, calcineurin A, Erk1/2 and TGF $\beta$ 1 protein levels [103], thereby associating these compounds with anti-hypertrophic, anti-fibrotic, anti-

inflammatory and antioxidant properties. Finally, antioxidants may also be considered for preventing remodelling of the RV as a result of pulmonary hypertension as phytophenol pterostilbene together with hydroxypropyl- $\beta$ -cyclodextrin were shown to reduce production of NOX-dependent superoxide anions and oxidative stress and preserve systolic function [104].

Apart from modulating pro-hypertrophic signalling pathways, antioxidants have also been found to improve mitochondrial function. In addition to preventing accumulation of ROS and suppressing ROS-dependent pro-hypertrophic MAPK and Akt signalling pathways, lycopene, a carotenoid antioxidant, was reported to prevent opening of the mitochondrial permeability transition pore and improve mitochondrial function, although its main mode of action was likely mediated through restoration of antioxidant response element activity and subsequent activation of antioxidant genes [105]. In other studies, isosteviol sodium which is a derivative of stevioside, a constituent of *Stevia rebaudiana*, which is commonly used as a noncaloric sugar substitute, has been reported to suppress the hypertrophic response in addition to restoring mitochondrial membrane potential and decreasing fission proteins (Fis1 and Drp1) [106] with accompanying decreases in ROS and elevated antioxidant levels (thioredoxin 1 and peroxiredoxin 2). Finally, the polyphenolic compound resveratrol was found to maintain normal LV volumes and preserve systolic function in the setting of cardiolipin deficiency-induced cardiac hypertrophy [107], which was attributed to improvements in mitochondrial respiration as well as decreased ROS levels and oxidative damage to the myocardium.

Based on these studies, it is clearly evident from several models that antioxidants do exert protection by negatively impacting several pathways that regulate hypertrophic growth, fibrosis, inflammation and oxidative stress to attenuate the disease phenotype while improving cardiac function. However, in a double-blind, randomized, single-centre pilot study involving HCM patients, high-dose NAC treatment for 12 months was found to have small effects on indices of cardiac hypertrophy and fibrosis when compared to placebo group [108]. While these findings were unexpected, given that NAC had previously been shown to exert protection in several models of cardiac hypertrophy [109, 110], the disparate observations between animal and human studies could be attributed to the genetic heterogeneity of patients with HCM as well as differential redox status.

In summary, while antioxidants have been shown to be protective in several animal models of cardiac hypertrophy, understanding their composition and pleiotropic functions is a critical prerequisite, as administration of such compounds in the clinical setting could result in inconsistent outcomes that could be attributed to disease aetiology and patient heterogeneity (see Table 1 for summary).

### **Selenoproteins**

The endoplasmic reticulum-resident selenoproteins are rapidly emerging as cardioprotective agents, as several studies have linked their dysfunction with susceptibility to oxidative stress and cardiovascular disease [111]. The involvement of selenoproteins in improving cardiac function in the setting of cardiac hypertrophy is

less studied, however, recent studies have associated these proteins with favourable effects. In one study, pre-treatment of H9c2 cells with H<sub>2</sub>S, was found to attenuate H<sub>2</sub>O<sub>2</sub>-induced cell death, oxidative stress and cell enlargement by activating the SCLY/H<sub>2</sub>Se signalling cascade which in turn increased expression and activities of selenoproteins (glutathione peroxidase and thioredoxin reductase) [112]. As H<sub>2</sub>S is endogenously produced by cystathionine gamma-lyase, mice deficient for this enzyme were found to display perturbed SCLY and selenoprotein P expression, though this effect on cardiac architecture or function was not investigated. While it would seem that both H<sub>2</sub>S and H<sub>2</sub>Se may exert protection through specific activation of selenoproteins, the increase in Nrf2-target genes post-treatment may suggest improvements to a broader range of antioxidants. In support, when Dahl salt-sensitive rats were provided with high-salt diet, the myocardial H<sub>2</sub>S pathway was found to be downregulated with elevated oxidative stress characterised by increased hydroxyl radicals, malondialdehyde and oxidised glutathione in addition to reduced total antioxidant capacity, carbon monoxide, catalase, glutathione, glutathione peroxidase and superoxide dismutase activities. Consistently, H<sub>2</sub>S was able to normalise these levels with accompanying inhibition of cardiac hypertrophy [113]. In other studies, apart from attenuating the hypertrophic phenotype, administration of exogenous NaHS was found to exert broad protection in a SIRT3-dependent manner by increasing total antioxidant capacity and superoxide dismutase activity while reducing malondialdehyde and superoxide levels [114]. Interestingly, NaHS also had an effect on mitochondrial shaping proteins, as evidenced by increased Opa1 and decreased Drp1 levels. A similar observation was made in another study where NaHS treatment resulted in increased expression of mitochondrial fusion proteins (Opa1, Mfn1 and Mfn2) and decreased expression of fission proteins (Drp1 and Fis1) with accompanying improvements in mitochondrial function, permeability potential, ultrastructure and increased mitochondrial numbers [115]. These observations suggest H<sub>2</sub>S to be pleiotropic as well, exerting a multitude of beneficial effect in the setting of cardiac hypertrophy, although enhancement of antioxidant capacity and suppression of oxidative stress may likely be its primary mode of action.

### ***Alternative therapies***

A cathelicidin-related antimicrobial peptide (CRAMP) has been shown to be cardioprotective in the setting of myocardial ischemia/reperfusion injury [116]. Similarly, CRAMP was found to protect against pressure overload by reducing the inflammatory response and oxidative stress [117]. Interestingly, CRAMP exhibited cardiomyocyte specificity as it was able to inhibit the hypertrophic response and oxidative stress in cardiomyocytes but not in endothelial cells. Mechanistically, CRAMP was reported to exert its anti-hypertrophic effects by activating the IGFR1/PI3K/Akt pathway through direct binding to IGFR1 while its antioxidative effects were a result of TLR9/AMPK $\alpha$  activation. In other studies, an analogue of the  $\alpha$ -calcitonin gene-related peptide ( $\alpha$ -CGRP) has been found to preserve heart function and prevent adverse cardiac remodelling and apoptosis in the setting of cardiac hypertrophy, highlighting the therapeutic potential of targeting the CGRP pathway

[118]. Moreover, when considering therapeutic strategies for HCM, the sphingosine-1-phosphate receptor modulator, fingolimod has been shown to improve diastolic function in the Tm-E180G mouse model of HCM [119], where mechanistic studies attributed these improvements to decreased S-glutathionylation of myosin-binding protein C and reduced oxidative stress via downregulation of NOX2. Though there was no change observed in the level of fibrosis, modulation of sphingolipid signalling may be a potential strategy for improving cardiac function in HCM patients.

Isolevuglandins (isoLGs) belong to a family of extremely reactive electrophiles and are generated by free radical-induced lipid oxidation and rearrangement of endoperoxide intermediates of the isoprostane pathway [120]. Considering the association between oxidative stress and cardiac hypertrophy, it is not surprising that an increase in isoLG protein adducts have been observed in cardiac tissue in the setting of pressure overload [121]. Interestingly, the IsoLG scavenger 2-hydroxybenzylamine and its less reactive isomer 4-hydroxybenzylamine were shown to attenuate LVH, reduce cardiac fibrosis and improve cardiac function, although the mechanisms remain unclear. In other studies, the analogues of imine stilbene (a compound that has previously been reported to exert cardioprotection and anti-aging via modulation of SIRT1) have also been found to alleviate the hypertrophic phenotype by suppressing several terminal stress kinases pathways [122]. It can be speculated that the imine stilbene analogue (compound 3e) is able to maintain mitochondrial homeostasis through reduction in oxidative stress, as it was found to prevent H<sub>2</sub>O<sub>2</sub>-mediated apoptosis.

Cell therapy approaches can also be considered for mitigating cardiac hypertrophy as remote transplantation of human adipose-derived stem cells (hADSCs) into the right hamstring of spontaneously hypertensive rats was found to attenuate cardiac hypertrophy and fibrosis with accompanying reductions in myocardial ROS [123]. Interestingly, hADSCs pre-treated with n-butylidenephthalide (BP) were shown to reduce cardiac hypertrophy and ROS more profoundly than naïve hADSCs while on the molecular level this resulted in increased STAT3 phosphorylation with subsequent activation and nuclear translocation which likely induced elevation of myocardial interleukin 10 (anti-inflammatory) levels and M2 macrophage polarisation. This study demonstrates that transplantation of BP-treated hADSC could be considered as a strategy to reduce cardiac hypertrophy even at an established phase of hypertension. Similarly, gene therapy strategies could also be considered to counteract development of cardiac hypertrophy in the setting of pressure overload as selective high-density lipoprotein-raising human apolipoprotein A-I gene transfer was reported to reduce pathological cardiac remodelling, nitro-oxidative stress and apoptosis, while increasing myocardial capillary density and improving cardiac function [124].

Finally, treatment with intraperitoneal injection of hydrogen was shown to prevent cardiac hypertrophy and improve cardiac function through suppression of NOX expression and inhibition of mitochondrial membrane potential depression which in turn blocked ROS-sensitive Erk1/2, p38 MAPK and Jnk signalling pathways [125]. In other studies, the anion nitrite has also been associated with beneficial properties

by improving antioxidant capacity in hypertensive rats, where a low nitrite dose was found to suppress ROS production, prevent activation of the mTOR pathway and attenuate cardiac hypertrophy, but without affecting blood pressure [126]. Conversely, maternal exposure to nitrogen dioxide was found to induce cardiac hypertrophy in male offspring with accompanying elevation of cardiac injury markers and calcineurin activity that were attributed to the cardiac-specific transcription factor Csx/Nkx2.5 whose expression was regulated by a ROS/HIF1 $\alpha$  axis and DNA hypomethylation modification at its promoter [127].

In summary, apart from antioxidants and selenoproteins, alternative therapies that can attenuate cardiac hypertrophy and improve cardiac function through suppression of oxidative stress is an exciting prospect although the specificity of such compounds should be investigated further.

### ***Drug repurposing***

Drug repurposing is a strategy for identifying new uses of approved or investigational compounds that are outside the scope of the original medical indication. This strategy offers various advantages over developing an entirely new drug for a given indication. Statins are the most common cholesterol-lowering drugs that have been reported to improve morbidity and mortality in high risk patients with cardiovascular disease [128]. Statins are proposed to have pleiotropic effects and this has been observed in mice following infusion of angiotensin II where atorvastatin and pravastatin were shown to attenuate cardiomyocyte hypertrophy and fibrosis in wild-type mice but not in SmgGDS<sup>+/-</sup> mice, suggesting that statins function through small GTP-binding protein GDP dissociation stimulator which regulated Rac1 expression, Erk1/2 activity, Rho-kinase activity and inflammatory cytokine secretion [129]. In another study that compared the effects of the sleep aid supplement, melatonin, in the setting of pathological and physiological cardiac hypertrophy, melatonin was found to attenuate oxidative stress and cardiac hypertrophy and improve cardiac function in the former but not in the latter [130]. Interestingly, melatonin was reported to signal through the retinoid-related orphan nuclear receptor- $\alpha$  (ROR $\alpha$ ) which was found to be otherwise decreased in human and murine pathological hypertrophic cardiomyocytes. Mechanistically, the beneficial effects of melatonin were attributed to transactivation of MnSOD by binding of ROR $\alpha$  to its response element located at the MnSOD promoter region. Long-term treatment with melatonin has also been reported to attenuate cardiac hypertrophy, slow the deterioration of cardiac contractile function and improve survival rate by activating PGC1 $\beta$  and suppressing oxidative stress in the setting of pressure overload [94].

The effect of enalapril, a drug used to treat hypertension has also been investigated in the setting of ageing-related cardiac hypertrophy [131]. Interestingly, late-life enalapril treatment of aged rats resulted in attenuated cardiac hypertrophy and oxidative stress-related molecular damage with accompanying increase in mitochondrial antioxidant defences. Intriguingly, enalapril seemed to have a profound effect on the level of the mitochondria as evidenced by increased mitochondrial mass and biogenesis as well as up-regulated mitochondrial fusion signalling and autophagy.

In other studies, celecoxib is a nonsteroidal anti-inflammatory drug that is used to treat arthritis amongst other conditions. Interestingly, in the setting of pressure overload, celecoxib was shown to attenuate cardiac hypertrophy and cardiac dysfunction by inhibiting apoptosis and inflammation via the MDM2/p53 pathway and the Akt/mTOR/NF- $\kappa$ B pathways respectively, in addition to suppressing oxidative stress via increases in Nrf2-mediated gene expression of multiple antioxidants [132]. Moreover, the traditional Indian Ayurveda medicine Yogendra Ras (YDR) has also been found to mitigate cardiac hypertrophy, which was attributed to the restoration of redox homeostasis as evidenced by suppression of biomarkers of oxidative stress and inflammation post-treatment [133]. Finally, cilostazol which is commonly used in the management of peripheral vascular disease [134] was shown to reduce nicotine-induced cardiomyocyte hypertrophy by enhancing cathepsin B activity which in turn resulted in improved autophagy flux and suppression of oxidative stress pathways [135].

In summary, while drug repurposing certainly seems an attractive prospect, caution is advised as certain compounds though beneficial for specific pathologies may exacerbate others. This has been observed with the PPAR $\gamma$  agonist rosiglitazone (RSG) which is used to treat diabetes but has also been shown to stimulate cardiac hypertrophy and oxidative stress. Mechanistically, this has been linked to secretion of miR-200a from adipocytes which in turn was found to decrease tuberous sclerosis protein 1 and activate mTOR in cardiomyocytes thereby inducing a hypertrophic phenotype [136]. This study highlights the crosstalk between adipocytes and cardiomyocytes in regulating adverse cardiac remodelling.

## **CONCLUSION**

Several studies have identified oxidative stress as a critical mediator of cardiac hypertrophy and consistently, targeting of oxidative stress has been shown to alleviate the hypertrophic response. Though initially thought to solely arise from pathways regulated by NADPH oxidase, xanthine oxidase and the mitochondrial electron transport chain, recent evidence has linked oxidative stress to metabolic disturbances, mitochondrial dysfunction, inflammation, impaired/overactive autophagy, dysregulated protein quality control and miRNA expression as well as impaired ion channel activity. Paradoxically, targeting of oxidative stress through the use of antioxidants in the clinical setting has yielded inconsistent results and diverse speculations have been made with regard to these unexpected findings including antioxidants being unable to reach the target cells in addition to not all ROS being detrimental and hence, targeting of ROS could induce a harmful reduced state. Here, we highlight that oxidative stress is indeed a complex process and while antioxidants may be beneficial in experimental settings where the disease model is well established, in the clinical setting where disease pathophysiology is confounded by different aetiologies and genetic heterogeneity, the use of antioxidants with undefined chemical composition could be inefficient or even harmful. Moreover, apart from general markers of oxidative stress (e.g. protein carbonylation, lipid peroxidation) more studies are needed to determine the specific proteins impacted by oxidative

modifications (e.g. sarcomere proteins, ion channels) as this will form the impetus for identifying and developing more precise therapeutic interventions which could potentially lead to better clinical outcomes in patients with cardiac hypertrophy.

## **FUNDING SOURCES**

Chrishan Ramachandra is supported by the Singapore Ministry of Health's National Medical Research Council under its Open Fund-Young Individual Research Grant (NMRC/OFYIRG/0073/2018), the National Health Innovation Centre Singapore under its Innovation to Develop Grant (NHIC-I2S-1811007) and the SingHealth Duke-NUS Academic Medical Centre under its SingHealth Duke-NUS Academic Medicine Research Grant (AM/TP033/2020 [SRDUKAMR2033]). Derek Hausenloy was supported by the British Heart Foundation (CS/14/3/31002), the Duke-NUS Signature Research Programme funded by the Ministry of Health, Singapore Ministry of Health's National Medical Research Council under its Clinician Scientist-Senior Investigator scheme (NMRC/CSA-SI/0011/2017), Centre Grant, and Collaborative Centre Grant scheme (NMRC/CGAug16C006). This article is based upon work from COST Action EU-CARDIOPROTECTION CA16225 supported by COST (European Cooperation in Science and Technology).

## ABBREVIATIONS

4E-BP1	Eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1
Akt	Protein kinase B
AMPK	AMP-activated protein kinase
ASK1	Apoptosis signal-regulating kinase 1
BCL10	B-cell lymphoma/leukemia 10
BIM	Bcl-2-like protein 11
BP	n-butylidenephthalide
BRD4	Bromodomain-containing protein 4
C1QTNF1	Complement C1q tumour necrosis factor-related protein 1
CaMKII	Calmodulin-dependent protein kinase II
cAMP	Cyclic adenosine monophosphate
CARD9	Caspase recruitment domain family member 9
CDC20	Cell division cycle protein 20 homolog
CHCHD3	Coiled-coil helix coiled-coil helix domain-containing protein 3
CHOP	C/EBP homologous protein
CRAMP	Cathelicidin-related antimicrobial peptide
CTRP3	C1q-tumour necrosis factor-related protein 3
CTRP9	C1q-tumour necrosis factor-related protein 9
Drp1	Dynamin-1-like protein
EGFR	Epidermal growth factor receptor
EMP	Endothelial microparticles
ENDO G	Endonuclease G
Erk1/2	Extracellular signal-regulated kinase 1/2
ERR $\alpha$	Estrogen-related receptor $\alpha$
FGF21	Fibroblast growth factor 21
Fis1	Mitochondrial fission 1 protein
FNDC5	Fibronectin type III domain containing 5
FOXO1	Forkhead box O1
FOXO3a	Forkhead box O3
Gb3	Globotriaosylceramide
GLA	Alpha-galactosidase
GLP-1	Glucagon-like peptide 1
GSK3 $\beta$	Glycogen synthase kinase 3 beta
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
H <sub>2</sub> S	Hydrogen sulfide
H <sub>2</sub> Se	Hydrogen selenide
hADSCs	Human adipose-derived stem cells
HDAC4	Histone deacetylase 4
hESCs	Human embryonic stem cells
HFD	High-fat diet
HIF1 $\alpha$	Hypoxia-inducible factor 1 alpha
HO-1	Heme oxygenase 1
Hsp22	Heat shock protein 22

IGFR1	Insulin-like growth factor 1
IL-6	Interleukin 6
isoLGs	Isolevuglandins
JAK2	Janus kinase 2
Jnk1/2	c-Jun N-terminal kinases 1/2
LC3	Microtubule-associated proteins 1A/1B light chain 3B
LKB1	Loss of tumor suppressor liver kinase B1
LOX	Lysyl oxidase
LVH	Left ventricular hypertrophy
MAPK	Mitogen-activated protein kinase
mdivi-1	Mitochondrial division inhibitor 1
MDM2	Murine double minute 2
Mfn1	Mitofusin 1
Mfn2	Mitofusin 2
MICOS	Mitochondrial contact site and cristae organising system
miRNA	Micro RNA
mitoKATP	Mitochondrial ATP-sensitive potassium channel
MitoQ	mitochondria-targeted ubiquinone
MK2	MAPK-activated protein kinase 2
MLP	Muscle LIM protein
MnSOD	Manganese superoxide dismutase
mtDNA	Mitochondrial DNA
MTG1	Mitochondrial ribosome associated GTPase 1
mTOR	Mammalian target of rapamycin
NAC	N-acetyl cysteine
NaHS	NaHS
NCOA4	Nuclear receptor coactivator 4
NF- $\kappa$ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHE1	Na <sup>+</sup> /H <sup>+</sup> exchanger isoform 1
NKA	Na <sup>+</sup> /K <sup>+</sup> -ATPase
NOX2	NADPH oxidase 2
NOX4	NADPH oxidase 4
NOX5	NADPH oxidase 5
Nrf2	Nuclear factor erythroid 2-related factor 2
Opa1	Dynammin-like 120 kDa protein, mitochondrial
p47 <sup>phox</sup>	Neutrophil cytosol factor 1
PDE	Phosphodiesterase type 1
PDK4	Pyruvate dehydrogenase kinase 4
PGC1 $\alpha$	Peroxisome proliferator activated receptor gamma coactivator 1 alpha
PGC1 $\beta$	Peroxisome proliferator activated receptor gamma coactivator 1 beta
PI3K	Phosphoinositide 3-kinase
PIKfyve	FYVE finger-containing phosphoinositide kinase
PKA	Protein kinase A
PLIN5	Perilipin 5

PP2A	Protein phosphatase 2A
PP2A $\alpha$	Protein phosphatase 2A catalytic subunit alpha
PPAR $\alpha$	Peroxisome proliferator-activated receptor alpha
PPAR $\gamma$	Peroxisome proliferator-activated receptor gamma
PPAR $\delta$	Peroxisome proliferator-activated receptor delta
Rac1	Ras-related C3 botulinum toxin substrate 1
RIP140	Receptor-interacting protein of 140 kDa
ROCK-2	Rho-associated coiled-coil containing protein kinase 2
ROR $\alpha$	Retinoid-related orphan nuclear receptor- $\alpha$
RPS6	Ribosomal Protein S6
RS-LPS	Lipopolysaccharide from Rhodobacter sphaeroides
SAM50	Sorting and assembly machinery 50
SCAD	Short-chain acyl-CoA dehydrogenase
SCLY	Selenocysteine lyase
SFXN1	Sideroflexin1
SIKE	Suppressor of IKK-epsilon
SIRT1	Sirtuin 1
SIRT3	Sirtuin 3
SIRT4	Sirtuin 4
STAT3	Signal transducer and activator of transcription 3
TAK1	Transforming growth factor beta-activated kinase 1
TBK1	TANK-binding kinase 1
TGF $\beta$ 1	Transforming growth factor beta 1
TIM50	Translocase of inner mitochondrial membrane 50
TLR4	Toll-like receptor 4
TLR9	Toll-like receptor 9
TRAF6	Tumour necrosis factor receptor-associated factor 6
TRPC3	Transient receptor potential channel, canonical 3
VDAC1	Voltage-dependent anion-selective channel 1
$\alpha$ -CGRP	Calcitonin gene-related peptide

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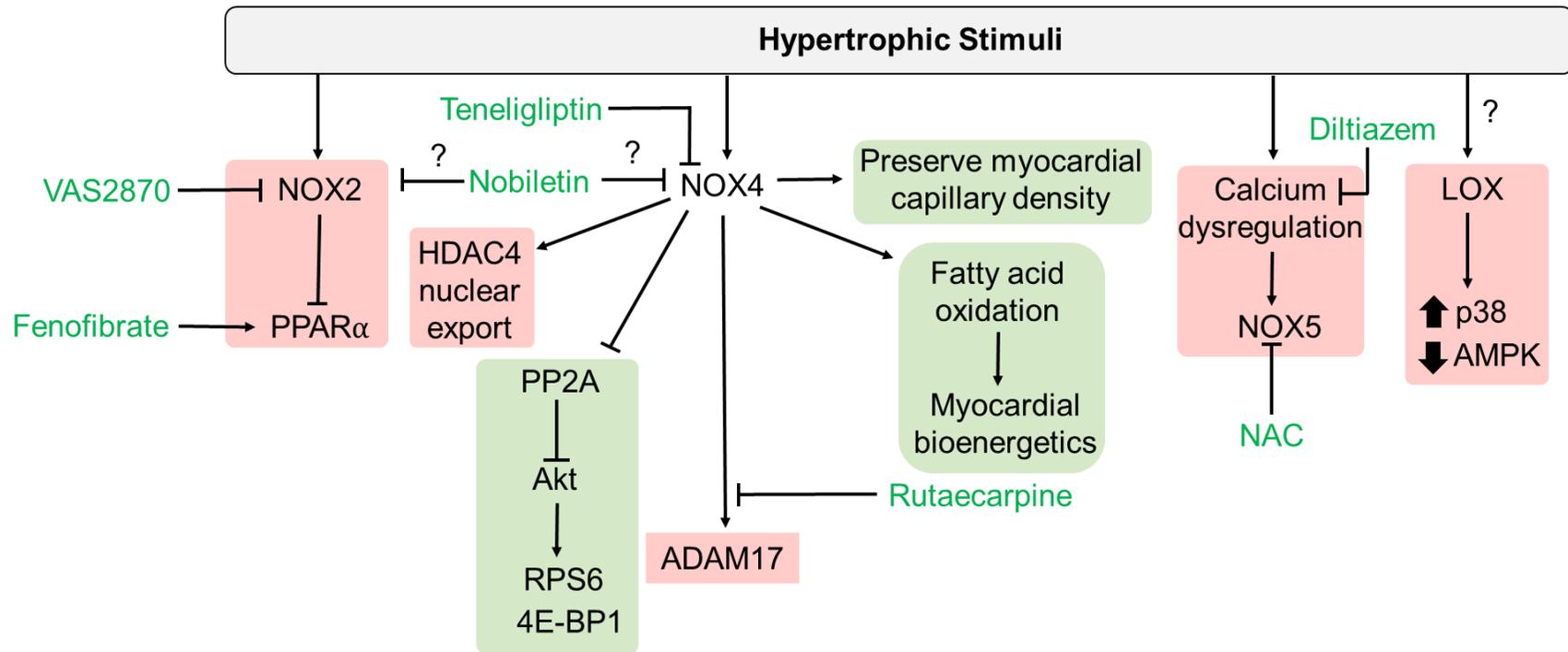
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<b>Table 1:</b> List of studies incorporating antioxidants as therapeutic intervention							
<b>Study type</b>	<b>Disease</b>	<b>Study participants (intervention group)</b>	<b>Intervention</b>	<b>Follow-up</b>	<b>Outcomes</b>	<b>Result</b>	<b>Reference</b>
Randomised clinical trial	CHD	144 (72)	1-Deoxynojirimycin (Mulberry)	4 weeks	Increased LVEF and reduced LVMI, aortic distensibility and atherosclerosis index ( $p < 0.05$ )	Positive	[137]
Randomised clinical trial	CHD	110 (56)	Chitosan Oligosaccharides	6 months	Improved Revised Cardiac Risk Index scores, quality of life scores and LVEF values	Positive	[138]
Randomised clinical trial	AMI	144 (73)	Coenzyme Q10	24 weeks	Attenuated LV remodelling: decreased wall thickness at and opposite the infarct site, decreased LVM, reduced end diastolic and systolic volumes	Positive	[139]
Randomised clinical trial	HCM	42 (29)	N-Acetylcysteine	12 months	Small effect on indices of cardiac hypertrophy: clinical phenotype, echocardiographic, and cardiac magnetic resonance imaging, function	Neutral	[108]
Randomised clinical trial	IC	200 (100)	Soybean isoflavones	24 weeks	Associated with the reduction of flow-mediated dilatation impairment at 24 weeks (odds ratio 0.30, 95% CI 0.14–0.85, $P = 0.01$ )	Positive	[140]
Randomised clinical trial	HF	100 (50)	Arjuna extract	12 weeks	No change in LVEF ( $24.3 \pm 7.1$ versus $25.5 \pm 7.7\%$ ; $p = 0.4$ ) or secondary outcomes (NYHA functional class level, distance covered in 6 min walk test, quality of life scores)	Neutral	[141]

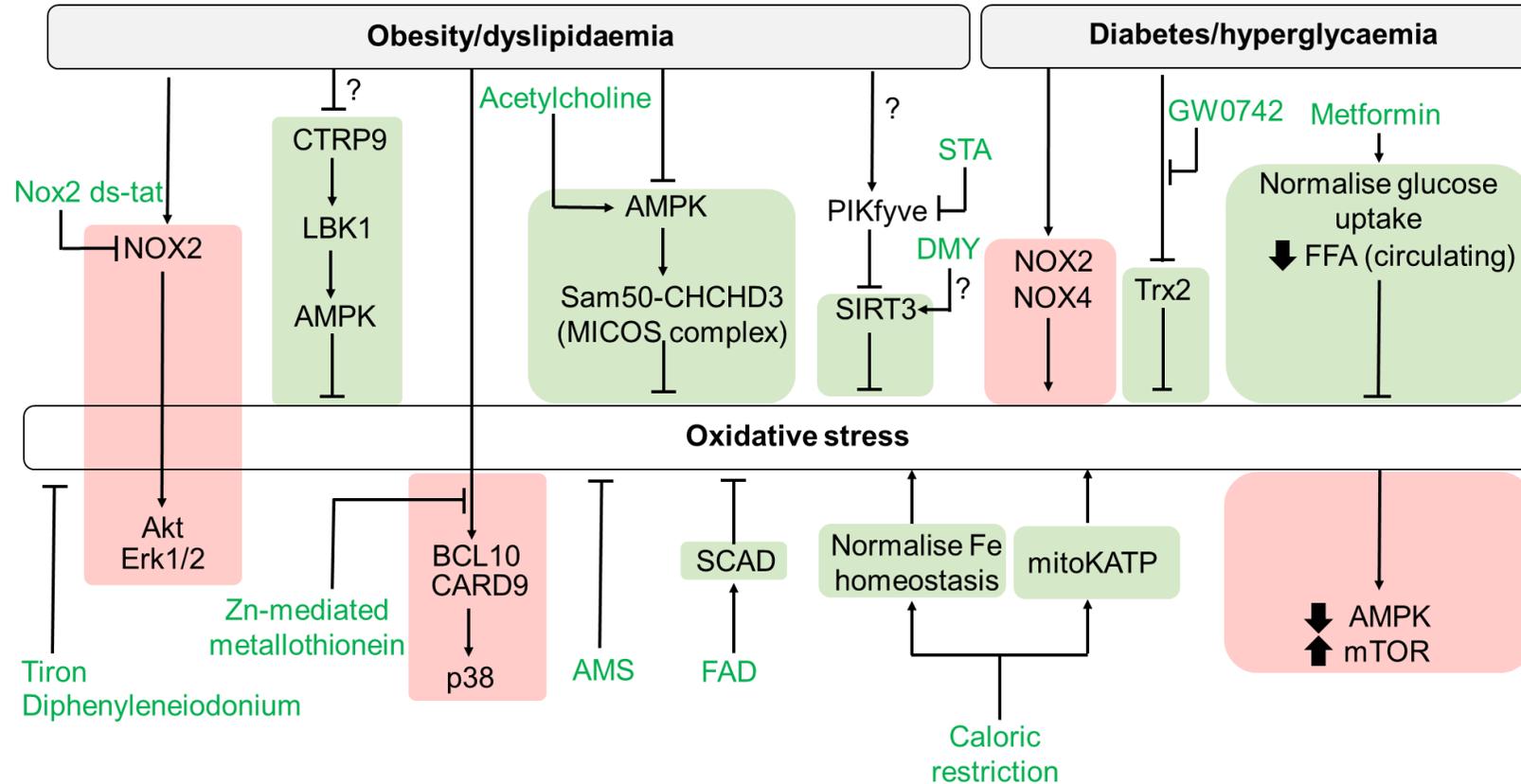
Randomised clinical trial	HF	420 (202)	Coenzyme Q10	2 years	The primary long-term endpoint (MACE) was reached by 15% of patients in the intervention group (HR: 0.50; 95% CI: 0.32 to 0.80; p=0.003). CoQ10 group also had low cardiovascular mortality and incidence of hospital stays for HF	Positive	[142]
Randomised clinical trial	STEMI	20 (10)	Manganese dipyridoxyl diphosphate (MnDPDP, catalytic)	48 hours	Failed to decrease plasma biomarker release, but decreased the mean infarct size and increased LVEF	Neutral	[143]
Prospective cohort study	CVD	32,561(all women)	Antioxidant-containing foods	10 years	Myocardial infarction hazard ratio for women comparing the highest quintile of dietary total antioxidant capacity to the lowest was 0.80 (95% CI, 0.67-0.97; p-trend = 0.02), but with no significant inverse association	Neutral	[144]
Prospective cohort study	CVD	98,469 (60,289 women)	Flavonoid	7 years	Men and women with total flavonoid intakes in the top quintile had a lower risk of fatal CVD (RR: 0.82; 95% CI: 0.73, 0.92; p-trend = 0.01). Five flavonoid classes (anthocyanidins, flavan-3-ols, flavones, flavonols, proanthocyanidins) were individually associated with lower risk of fatal CVD	Positive	[145]
Randomised clinical trial	HF	39,815 (19,913, all women)	Vitamin E	10 years	No significant effect on HF risk (HR 0.93; 95% CI, 0.71–	Neutral	[146]

					1.21; p=0.59). Vitamin E was inversely related to development of HFpEF (HR 0.59; (95% CI, 0.38–0.92; p=0.02), but there was no statistically significant effect on the risk of developing systolic HF (HR 1.26; 95% CI, 0.84–1.89; p=0.26)		
Randomised clinical trial	HF	74 (38)	Allopurinol	4 weeks	No additional beneficial effects of alopurinol were found after completion with the short-term atorvastatin treatment in HF patients	Neutral	[147]
<b>Abbreviations:</b> AMI- acute myocardial infarction; CHD- coronary heart disease; CVD- cardiovascular disease; HCM- hypertrophic cardiomyopathy; HF- heart failure; IC- ischemic cardiomyopathy; STEMI- ST-segment elevation myocardial infarction; LVMI- left ventricular mass index; LVM – left ventricular mass; MACE- major adverse cardiovascular events; HFpEF- heart failure with preserved ejection fraction							

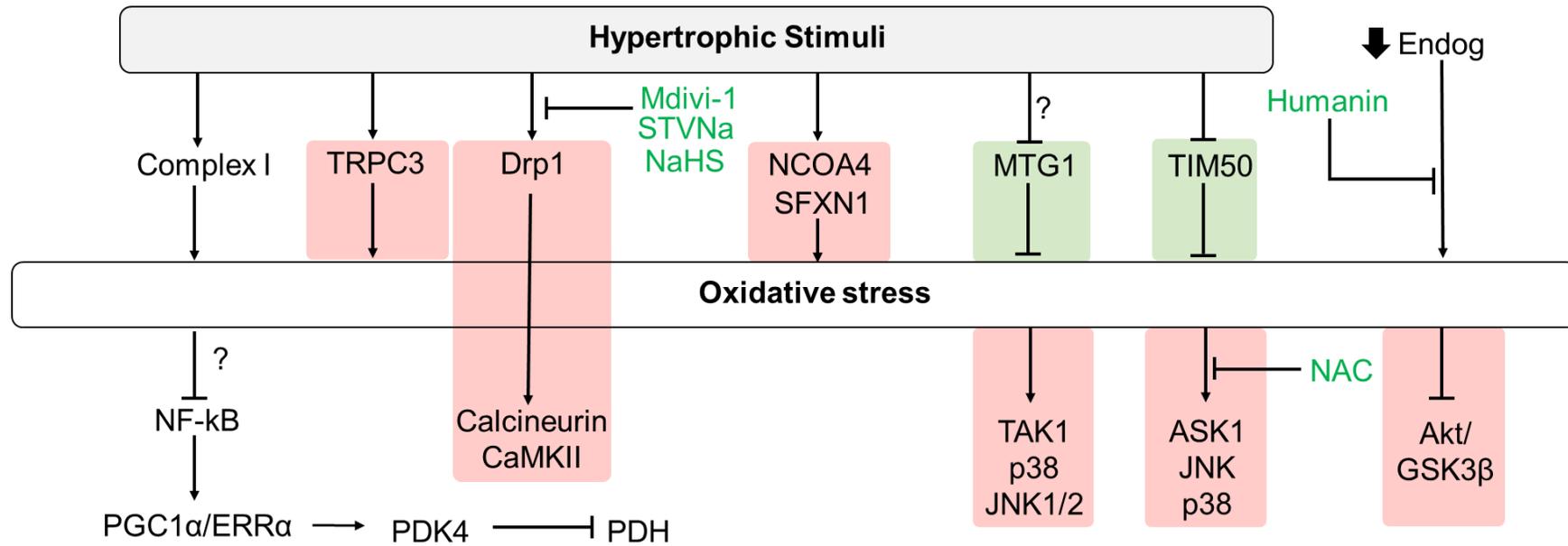
**Figure 1:** Schematic indicating oxidative stress pathways regulated by NOX isoforms with potential points of intervention (Red boxes- detrimental cascades; green boxes- protective cascades)



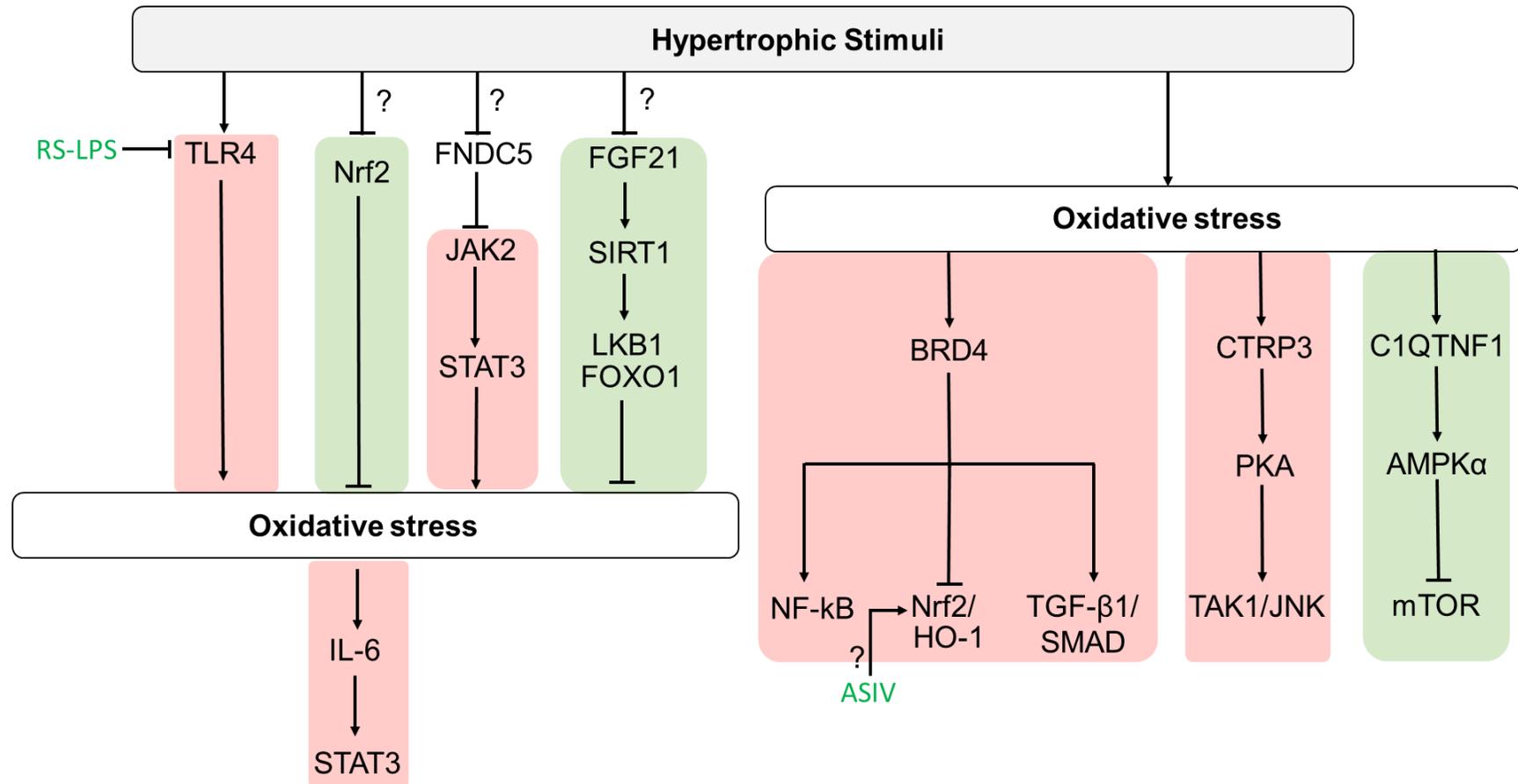
**Figure 2:** Schematic indicating oxidative stress pathways in response to metabolic disturbances with potential points of intervention (Red boxes- detrimental cascades; green boxes- protective cascades; ?- further validation required)



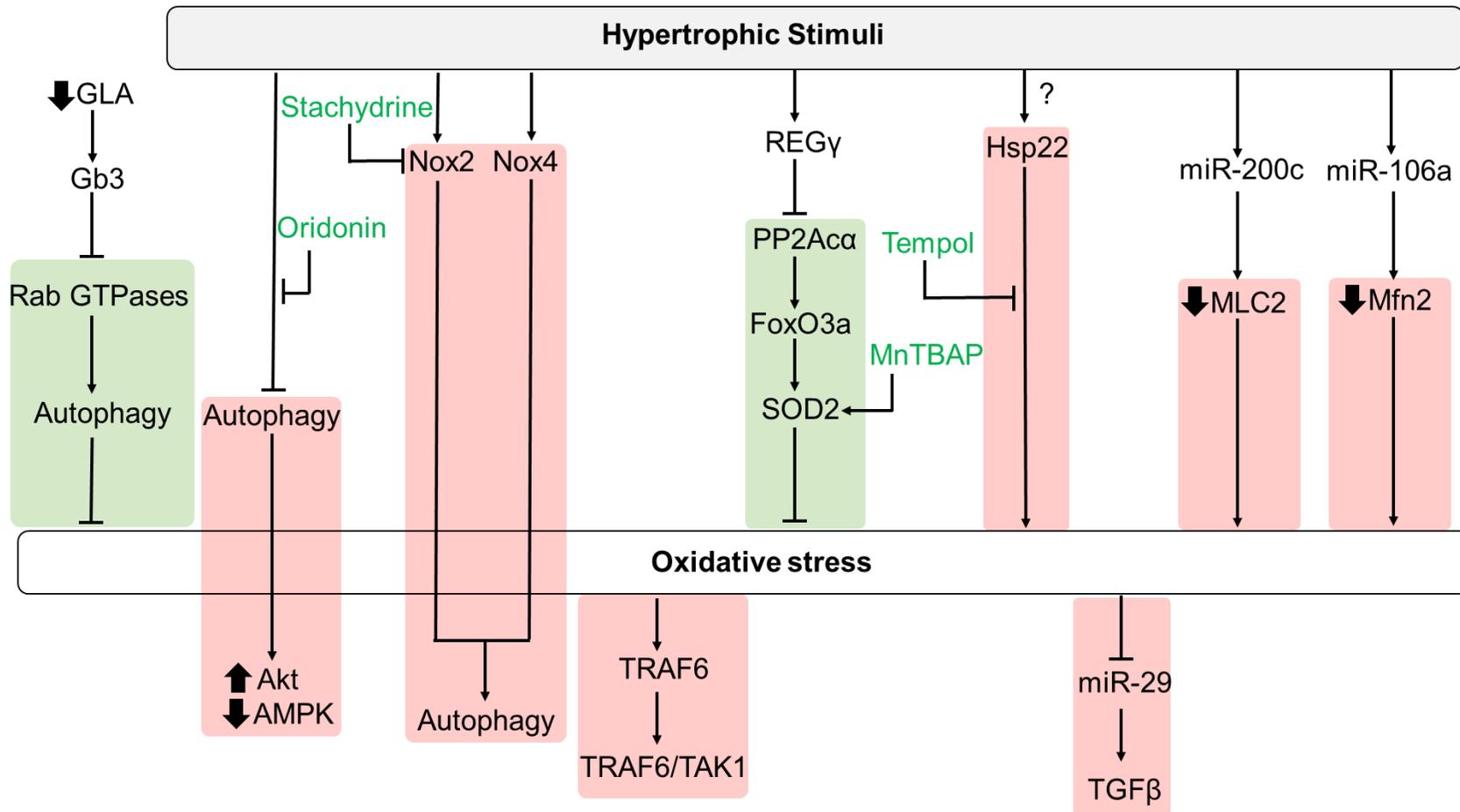
**Figure 3:** Schematic indicating oxidative stress pathways in response to mitochondrial dysfunction with potential points of intervention (Red boxes- detrimental cascades; green boxes- protective cascades; ?- further validation required).



**Figure 4:** Schematic indicating oxidative stress pathways in response to inflammation with potential points of intervention (Red boxes- detrimental cascades; green boxes- protective cascades; ?- further validation required).



**Figure 5:** Schematic indicating oxidative stress pathways in response to dysregulated cellular processes (impaired/excessive autophagy, dysregulated protein quality control and miRNAs) with potential points of intervention (Red boxes- detrimental cascades; green boxes- protective cascades; ?- further validation required).



**Figure 6:** Schematic indicating oxidative stress pathways in response to impaired ion channel activity with potential points of intervention (Red boxes- detrimental cascades; green boxes- protective cascades; ?- further validation required).

