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PATHOPHYSIOLOGICAL BASIS FOR NUTRACEUTICAL SUPPLEMENTATION IN HEART FAILURE: A COMPREHENSIVE REVIEW

From the Optimal Nutraceutical Supplementation in Heart Failure (Onus-HF) Working Group

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Abstract

Heart failure (HF) is a disease state which has been shown to affect 1-2% of the global population, being often associated with comorbidities such as diabetes, hypertension, obesity or hyperlipidaemia which increase the prevalence of the disease, the rate of hospitalization and the mortality. Although recent advances in both pharmacological and non pharmacological approaches have led to significant improvements in clinical outcomes in patients affected by HF, residual unmet needs remain. Treatment of the disease remains unclear particularly related to poorly defined strategies in the early stages of myocardial dysfunction.

Nutritional support in patients developing HF and nutraceutical supplementation have recently been shown to may contribute in the protection of the failing myocardium, though their place in the treatment of HF still needs to be better clarified.

In this context, the ONUS-HF working group aimed to assess the optimal nutraceutical approach to HF in the early phases of the disease in order to counteract selected pathways which are imbalanced in the failing myocardium.

In particular, we reviewed several of the most relevant pathophysiological and molecular changes occurring druing the early stages of myocardial dysfunction. These include mitochondrial and sarcoplasmic reticulum stress, insufficient nitric oxide (NO) release, cardiac stem cell mobilization and imbalanced regulation of metalloproteinases. Several candidates for nutraceutical supplementation in HF, such as CoQ10, grape seed extract, Olea Europea L- related antioxidants, SGLT2 inhibitors-rich apple extract and bergamot polyphenolic fraction have been assessed for their potential contribution to cardiomyocyte prottection.

This approach should define the optimal approach for more targeted and successful strategies based on the use of nutraceuticals in HF to be confirmed by means of clinical trials exploring efficacy and safety of these compounds.

Key words: Heart failure, dysfunctional cardiomiocytes, pathophysiological mechanisms, oxidative stress, nutraceuticals

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1. Introduction

Heart failure (HF) represents a multifactorial disease with a prevalence of 1-2% in the global population [1]. Comorbidities such as diabetes, hypertension, obesity or hyperlipidaemia are frequently associated with HF, thereby increasing the prevalence of the disease, the rate of hospitalization and the associated mortality [2,3]. In addition, ageing affects the number of patients affected by HF; the occurrence of the disease rises to 10% or more in people aged >70 years [4,5].

According to recent international treatment guidelines, better management of risk factors and increased pharmacological and non-pharmacological treatment significantly reduces the impact of HF and its consequences. In particular, recent data from a pilot study on HF performed by the European Society of Cardiology (ESC) showed that all-cause mortality at 12 months for hospitalized and stable/ambulatory HF patients was 17% and 7%, respectively, and the 12-month hospitalization rates were 44% and 32%, respectively [1]. Hence, several relevant outcomes of HF remain unsatisfactory and additional studies are required to address the current unmet needs in the management of this disease.

Recently, growing evidence suggests that greater control of nutritional balance in patients suffering HF (with special regard to micronutrients and nutraceutical supply) leads to significant improvement of symptoms and consequences of the disease [5,6,7]. On the other hand, identification of novel biomolecular mechanisms involved in the development of the HF, which are only partially counteracted by current treatments, suggests the potential for additional contributions to the management of HF via optimal micronutrient and nutraceutical supplementation [8,9,10,11,12].

Micronutrient deficiency can be considered a common feature in many HF patients. In fact, HF can be caused by some specific micronutrient deficiencies [13,14,15,16]. Increased susceptibility to the effects of micronutrient deficiencies by patients with HF may be due to the impairment of myocardial contraction and skeletal muscle mechanisms (possibly compounded by a vitamin D

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deficiency), and increased oxidative stress. On the other hand, multiple conditions related to micronutrient deficiency can lead to a depletion of lean body mass (LBM). A proinflammatory state, inadequate intake, metabolic changes, an increase in oxidative stress, and increased nutrient loss can all contribute to a loss of LBM. Furthermore, depletion of LBM can affect vital organs, including the myocardium; negatively influencing its functional capacity [12,17].

Although preliminary evidence suggests that nutraceutical supplementation could be beneficial in treating patients with impaired cardiac function, limited evidence exists supporting their extensive use in patients with HF as reported in recent studies and meta-analyses (Table 1).

Properties and most relevant evidences of nutraceutical effects in HF

Plant	Bioactive component	Properties	*In vitro/in vivo* models	Clinical trials	References
Brassicaceae family *Gramineae family*	Coenzyme Q10	Antioxidant and anti-inflammatory activity Key component of METC and in ATP production Bioenergetic effect ↑ p-AMPK ↑ Akt/eNOS activity ↑ HO-1 expression ↑ hemodynamic parameters ↑ LV function ↓ 3-NT ↓ MDA ↓ Nox2 gene expression ↑ Vasodilation ↓ Aldosterone levels ↑ Fatty acid oxidation ↑ VLDL ↓ LDLc/HDLc ↓TC/HDLc ↓ Fibrinogen ↓ SBP ↓ DBP	- EPCs - Isoproterenol- induced HF in rats - Diabetic cardiomyopathy in mice	-HFrEF -Hypertension -T2DM - MetS Hyperlipidei ma - MI	[18-30]
Citrus Bergamia Risso et Poiteau	BPF	↓ Serum glucose, TG, TC, LDL-C, VLDL-C ↑ HDL-C ↑ fecal sterol excretion Re-arrangement of lipoprotein particles ↑ Lipid transfer protein system ↓ pCEH ↑ SOD, catalase ↓ SMC proliferation, LOX-1, p-PKB ↓ ROS, TBARS, MDA, Nitrotyrosine ↑ LV function ↓ pathologic cardiac	- eCSCs - rat neointimal hyperplasia - hypercholesterolemic diet fed rats - Doxo-induced cardiotoxicity in rats	- Hyperlipemia - MetS - T2DM	[31-46]

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		remodelling ↓ detrimental autophagy ↓ apoptosis ↓ 8OHdG ↑ newly formed myocytes			
Oleaceae family (Olea europaea Linn.)	Oleuropein, hydroxytyrosol	↓ CK-MB, GSSG, TBARS, LDH ↓ MDA, 3-NT, ET-1, IL-1 β, IL-6, TNFα ↑ eNOS ↓ PCs, iNOS ↑ p-Akt, p-AMPK ↑Prdx-1 and Prdx-2 ↓ TC, TG ↑ SOD and GSH activity ↑ integrity of complex III of the METC ↓ infarct size ↓ myocyte apoptosis ↑ LV fuction ↑ pGS3K-β/GS3K-β ↑ Sirt-1, pFOXO3a ↓ myocardial fibrosis ↓ pMEK, pERK1/2, p53, p-IκBα ↑ pSTAT-3 ↓ CYP2E1, OH-1, NF-κB, COX-2 ↓ GRP78, CHOP	- VPCs - $CoCl_2$-induced hypoxia in H9C2 cells - ISO-induced MI in rats - myocardial I/R in rats - doxo-treated rats - myocardial I/R in hypercholesterolemic rabbits - myocardial infarction in rats - T2DM and renal hypertension in rats - cisplatin-induced kidney injury in mice		[47-64]
Malus Malus sieversii	Phlorizin	↑ endothelium-indipendent relaxation ↓ human ox-LDL ↓ postprandial blood glucose rise, HbA1c ↑ urinary glucose excretion ↓ urinary albumin excretion ↓ glycohemoglobin, insulin, TG ↑ muscle GLUT4 ↓ renal GLUT2 ↓ kidney epithelial vacuolization ↓ hepatic glucose production ↑ ketone bodies amount	- isolated rabbit coronary artery - high glucose diet in Std ddY mice - neonatally STZ-induced diabetic rats - STZ-induced diabetic rats		[64-77]
Vitis vinifera	Proanthocyanidins	free radical scavenging activity ↓ TBARS levels ↓ DNA fragmentation ↓ p53 ↑ Bcl-2, Bcl-XL ↓ liver toxicity and DNA fragmentation ↓ hepatocyte apoptosis and necrosis ↓ JNK-1, c-Jun ↓cardiomyocyte apoptosis ↑ post-ischemic cardiac function ↑ mitochondrial IDH, SDH, MDH, α-KGDH ↑ respiratory chain NADH dehydrogenase and CCO	- TPA-induced ROS /RNS in mice - human oral keratinocytes - AAP-induced liver injury in mice - myocardial I/R in rats		[78-94]

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Zygophyllaceae family (Tribulus terrestris L.)	ferulic acid, diosgenin, saponins	↑Total antioxidant activity ↑ SOD, GPx, CAT activity ↓ MDA ↓ LDH, CK-MB, SGOT, SGPT, Calcium ↑ cell viability ↑ Integrity of mitochondrial PTP ↑ Activity of mitochondrial respiratory complexes ↑ oxygen consumption rate ↑ ATP level ↓ HIF-1α ↑ Mitochondrial OPA1, Mfn1, Mfn2 ↓ Drp1 and Fis1 ↓ Bax, Bad ↑ Bcl-2, p-Akt ↓p-P38, p-JNK ↓ Heart rate ↓ cardiac fibrosis ↓ IL-6, TNFα, IL-1β, MCP-1 ↑ IL-10 ↓ Nuclear traslocation of NF-κB ↑ coronary artery dilation ↑ ECG	- Myocardial ischemia in H9C2 - ISO-induced ischemia in rats	- angina pectoris	[95]
Vitis vinifera	Resveratrol	↑ oxygen consumption rate ↑ SIRT1 and p-AMPK ↓ PCG1α acetylation ↑ PCG1α activity ↑ insulin sensitivity ↑ endothelial function ↑SBP ↑ adiponectin, IL-10 ↓ PAI-1, hsCRP, TNF α, IL-6 ↓ HbA1c, TC, TG ↓ number of angina episodes ↑ LV diastolic function ↓ ANP ↑ LC3II/LC3I ↓p-mTOR, p-p70S6K ↑myocardial ATP content ↓ cleaved-caspase-3	- C2C12 mouse myoblast cells - MEFs (mouse embrionic fibroblasts) - H9C2 cells - isolated gastrocnemius muscle - HFD treated mice - KKAy mice - MI in mice and rats - SHRs rats	- hypertension - CAD - T2DM - stable angina pectoris - MI	[96-106]

Table 1. BPF, Bergamot Polyphenolic Fraction; METC, Mitochondrial Electron Transport Chain; ATP, *Adenosine Tri-Phosphate; AMPK*, 5' Adenosine Monophosphate-activated Protein Kinase; eNOS, endothelial *Nitric Oxide Synthase; H0-1*, Heme oxygenase 1; LV, Left Ventricular; 3-NT, Nitrotyrosine; MDA, Malonildialdehyde; Nox2, NADPH oxidase-2; VLDL-C, Very Low Density Lipoprotein Cholesterol; LDL-C, Low Density Lipoprotein Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; TC, Total Cholesterol; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; TG, Triglycerides; pCEH, pancreatic Cholesterol Ester Hydrolase; SOD, Superoxide Dismutase; SMC, Smooth Muscle Cells; LOX-1, Lectin-type Oxidized LDL receptor 1; PKB, Protein Kinase B; ROS, Reactive Oxigen Species; TBARS, Thiobarbituric Acid Reactive Substances; 8OHdG, 8-Hydroxy-2′-deoxyGuanosine; GPx, Glutathione Peroxidase; CK-MB, Creatine kinase isoenzyme; GSSG, Glutathione disulfide; LDH, *Lactate Dehydrogenase; ET-1, Endothelin-1;* IL-1β,

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Interleukin-1β; IL, Interleukin; TNF-α, Tumor Necrosis Factor- α; PCr, Phospho-Creatine; iNOS, inducible Nitric Oxide Synthase; Prdx, *Peroxiredoxin; GSH, Glutathione;* GS3K-β, Glycogen Synthase Kinase-β; Sirt-1, Sirtuin-1; FOXO3a, Forkhead box O3a; ERK1/2, Extracellular signal-Regulated Kinase 1/2; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells Inhibitor-α; STAT-3, *Signal Transducer and Activator of Transcription 3;* CYP2E1, Cytochrome P450 Family 2 Subfamily E Member 1; NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells; COX-2, Cyclooxygenase-2; GRP78, Glucose-Regulated Protein 78; CHOP, C/EBP Homologous Protein; ox-LDL, oxidized-Low Density Lipoprotein; HbA1c, glycated Haemoglobin; GLUT, Glucose Transporter; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; JNK-1, c-Jun N-terminal Kinase; IDH, Isocitrate Dehydrogenase; SDH, Succinate Dehydrogenase; MDH, Malate Dehydrogenase; α-KGDH, *α*-Ketoglutarate Dehydrogenase; NADH, Nicotinamide Adenine Dinucleotide; CCO, Cytochrome C Oxidase; GPx, Glutathione Peroxidase, CAT, Catalase; SGOT, Serum Glutamic Oxaloacetic Transaminase; SGPT, Serum Glutamic Pyruvic Transaminase; PTP, *Permeability Transition Pore; HIF-1α, Hypoxia-Inducible Factor 1-α;* OPA1, Optic atrophy Protein-1; Mfn, Mitofusin 1; Drp1,Dynamin-related protein 1; Fis1, mitochondrial Fission 1 protein; Bax, Bcl-2-like *protein* 4; Bad, Bcl-2 associated agonist of cell death; MCP-1, Monocyte Chemoattractant Protein-1; ECG, *electrocardiogram;* PCG1α, Peroxisome proliferator-activated receptor gamma Coactivator 1 alpha; PAI-1, Plasminogen Activator Inhibitor; Hs-CRP, High sensitivity C-Reactive Protein; ANP, Atrial Natriuretic Peptide; LC3, Microtubule-associated protein 1A/1B-light chain 3 ; mTOR, mechanistic Target Of Rapamycin; EPCs, Endothelial Progenitor Cells; HF, Heart Failure; eCSCs, endogenous Cardiac Stem Cells; Doxo, Doxorubicin; VPCs, Vascular Progenitor Cells, ISO, Isoproterenol; MI, Myocardial Infarction; I/R, Ischemia/Reperfusion; T2DM, Type 2 Diabete Mellitus; STD, Streptozotocin; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; RNS, Reactive Nitrogen Species; AAP, acetaminophen; HFD, High Fed Diet; HFrEF, Heart Failure with reduced Ejection Fraction; MetS, Metabolic Syndrome; CHD, Chronic Heart Disease; CAD, Coronary Artery Disease.

In fact, data collected in patients with HF and reduced ejection fraction (HFrEF) suggest that nutraceutical supplementation is accompanied by a non-significant decrease in mortality and cardiovascular hospitalisations[107,108]. Many other studies were too small or underpowered to adequately appraise clinical outcomes [107, 108]. The major weaknesses in the existing evidence include the small number of studies performed, the small number of study participants, diminished

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study quality with a high risk of bias, and that most studies were performed before the widespread use of neuro-hormonal antagonists. Hence, definite conclusions cannot be drawn. Consequently, it is general opinion that clinicians should presently favour other treatments that have more clearly been shown to decrease mortality [107,109].

Therefore, although the use of nutraceutical supplementation is of interest in the prevention and treatment of cardiovascular disease, the potential for its use in the treatment of HF is still to be adequately assessed and supplementary studies are required to verify the optimal use of nutraceutical supplementation in counteracting myocardial dysfunction in HF patients.

The aim of this review article is to assess the pathophysiological mechanisms involved in the early stages of HF and the potential for timely nutraceutical supplementation to support the failing myocardial cells in tandem with the pharmacological and non-pharmacological interventions currently being used. Moreover, a further objective is to optimize nutraceutical intervention through the identification of candidate natural extracts to be used in clinical studies regarding patients undergoing HF.

2. Emerging pathophysiological mechanisms involved in the development of HF

2.1 Energy deficiency and mitochondrial impairment in HF

In patients with HF, mitochondrial dysfunction and subsequent energetic deficits are evident and can be detected both *in vitro* and *in vivo* very early in the course of the disease [110]. In particular, evidence has been accumulated via non-invasive measurement of the myocardial phosphocreatine (PCr), a very sensitive biomarker of the energetic balance of cardiomyocytes, as determined by means of 31P- magnetic resonance spectroscopy [111]. In particular, a reduction in the PCr to adenosine triphosphate (ATP) ratio indicates an energy shortage and predicts adverse outcomes in HF patients [112]. In particular, degradation of ATP, associated with diastolic dysfunction, represents a biomolecular target of HF with preserved ejection fraction (HFpEF) which is accompanied by multiple alterations in energy/mitochondrial relationship [113,114,115,116,117].

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Presently, important aspects of the energy starved heart concept are incompletely resolved; the cause or causes for this energetic deficit are still unclear. Previously and more recent studies [118,119,120,121] have suggested that, in failing versus non-failing hearts, the electron transport chain (ETC) function is not impaired [122]. Instead, Krebs cycle activity, appears to be impaired in failing hearts [123]. The substrate metabolism, which includes the capacities of glycolysis and fatty acid oxidation which provide acetyl-coenzyme A to the Krebs cycle, also appears to be impaired in failing hearts [124]. Furthermore, the degree to which the energetic deficit contributes to the contractile deficit is still unclear. The progressive expression of mitofusins (Mfn1 and Mfn2) may contribute to the deterioration of the well-organized spatial pattern of mitochondria in the cardiac myocytes. This progressive expression can also potentially affect the SR-mitochondrial calcium ions ($Ca^{2+)}$ microdomain [124,125,126,]. In fact, in mitochondria of failing human hearts, the open probability of the mitochondrial Ca^{2+} uniporter (MCU) is reduced [127].

Together, these data indicate that, in heart failure, a reduced mitochondrial Ca^{2+}uptake could impair the Ca^{2+}-induced stimulation of the Krebs cycle, impairing the regeneration of NADH and the reduced form of NADPH. According to the "redox-optimized reactive oxygen species (ROS) balance" (R-ORB), these are core mechanisms in human hearts for production of energy and activation of anti-oxidant enzymes [128], since in animals lacking a functional mitochondrial nicotinamide nucleotide transhydrogenase (Nnt) gene, oxidative stress and less systolic dysfunction are reduced as well as premature death did not occur [129]. Oxidation of NADH, an important consequence of this energy supply-and-demand mismatch, occurs during elevated cardiac workload. The depleted NADPH-coupled anti-oxidative capacity is then overwhelmed by ROS production by NADH-coupled respiration in the ETC. Consequently, this favours the reverse mode of the mitochondrial Nnt, thus dissipating the anti-oxidative capacity [129,130]. Hence, extreme oxidation, as aforementioned, and reduction of the mitochondrial redox state, such as that which occurs during ischaemia, are to be avoided in order to achieve optimal conditions for cardiac mitochondria. However, the working heart constantly produces adenosine diphosphate (ADP),

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physiologically accelerating respiration and increasing oxidation in the respiratory chain. Therefore, it is unlikely that increased oxidative stress in HF is due solely to a net increase of ROS production, but rather due to diminished ROS scavenging capacity [129]. This leads to a vicious circle, increasing oxidative stress by exacerbating the mismatch of energy supply and demand [131]. Mitochondria contain unsaturated fatty acids, iron sulphur clusters, densely packed proteins, and multiple copies of mitochondrial DNA (mtDNA), all of which are essential to mitochondrial function and all are typical targets of oxidative damage [132]. The most vulnerable to oxidative damage, due to their proximity of ROS production, are the ETC complexes, which include cardiolipin [133]. Oxidative stress triggers the peroxidation of cardiolipin, impairing cristae formation [134]. Moreover, the respirasome and the detachment of cytochrome c, a mobile electron carrier in the inner mitochondrial membrane (IMM), are also affected by peroxidation of cardiolipin [135]. Mitocondrial DNA is associated with the IMM and vulnerable to oxidative damage due to the lack of protective histones. Furthermore, damages to mtDNA result in ATP synthesis reduction, reduced ETC activity and a further increase in electron slippage to oxygen, thus setting up a feed-forward cycle of ROS-induced ROS production [136,137,138]. Hence, at the centre of an efficient cardioprotection strategy, in the early stages of HF, is the preservation of the functionality of mitochondria in cardiomyocytes, which represents a consistent challenge.

2.2 *Sarcoplasmic reticulum stress in HF*

Oxidative stress is a critical mechanism of cardiovascular diseases (CVDs) [139]. Endoplasmic reticulum (ER) stress, an important inducer of oxidative stress [140,141], is associated with multiple (CVDs), including hypertension, HF, and cardiac hypertrophy [142,143]. It is evidenced that oxidative stress can be regulated by ER and mitochondria. In particular, in models of heavy metal-induced myocardial dysfunction, an increase in the level of ER stress markers occurs. This suggests that excessive ER stress is associated with the mechanism of oxidative stress and heart dysfunction. Hence, the detrimental effects of heavy metal and other toxicants on the heart could be

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avoided by natural antioxidants. The mechanism of cardiprotection can possibly explain the reduction of ER stress by natural antioxidants. These act against cardiotoxic agents by regulating the ER-related pathway which may be associated with protein damage and intracellular Ca2+ anomalies [143, 144]. In particular, activated during stress conditions, protein kinase RNA-like ER kinase (PERK) is a mediator of eIF2α phosphorylation following folded protein exposure, thus preventing protein synthesis by selectively prompting Activating Transcription Factor 4 (ATF4) translation in the instantaneous response to ER stress [141,142]. Importantly, ER stress markers are involved in the PERK/eIF2α signalling pathway, which also promotes ER stress and contributes to organ injury induced through ER stress-mediated apoptosis [145,146,147]. Increased intracellular GRP78, considered to be a marker of unfolded protein response (UPR) and chaperone of ER stress, demonstrates unfolded and mis-folded protein aggregation in ER and UPR [148,149]. This effect is counteracted by natural antioxidants such as pro-anthocyanosides. Following ER stress [143,144], the transcription factor C/EBP homologous protein (CHOP), an important downstream target triggered by ATF4, is highly involved in apoptosis response. Toxins affecting cardiomyocyte functionality upregulate GRP78 to stimulate UPR and the PERK/eIF2α signalling pathway, inducing oxidative stress and apoptosis and ultimately contributing to apoptosis-mediated heart dysfunction. Conversely, natural antioxidants decrease ER stress markers, thus demonstrating that active ingredients of plant extracts can effectively inhibit the GRP78-mediated PERK/eIF2α signalling pathway, attenuate cardiomyocyte ER stress and revive heart function [145,149].

2.3 Imbalanced metalloproteinase regulation in HF

The extracellular cardiac matrix (ECM), a complex architectural network, maintains equilibrium between the deposition and degradation of matrix proteins by preserving the correct cardiac geometry and structural integrity of the myocardium [150]. The ECM aids heart cells to function properly by forming a specific scaffold which allows for the anchoring of these proteins [151]. The ECM turnover influences physiological and pathological processes, such as cell proliferation,

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differentiation and tissue morphogenesis [152]. The ECM is also liable for the transduction of mechanical force in the cardiac vessels and heart as well as for influencing the diastolic compliance of arterial walls [152]. Matrix metalloproteinases (MMPs) are enzymes capable of degrading ECM structural proteins and their endogenous tissue inhibitors of metalloproteinases (TIMPs). They are the main mediators of ECM remodelling [150]. These processes are tightly regulated under normal conditions. The role of MMPs and TIMPs is positive and negative in cardiac remodelling, with TIMP's controlling MMPs minimizing the degradation of the matrix [151,152]. The MMPs play a pathological and irreversible role in remodelling ECM which is important in both compensatory cardiac hypertrophy and acute decompensated heart failure [153]. MMPs also mediate the ventricular remodelling caused by myocardial infarction or viral myocarditis [153]. Several studies indicate that the serum level of MMP-2 is an independent predictor of mortality in HF patients [154].

There are five main groups of MMPs: collagenases, matrilysins, gelatinases, stromelysins, and membrane-type metalloproteinases/membrane-type MMPs; the divisions of these groups are based on their substrate specificity and structure [155]. Two of these, Gelatinase A and Gelatinase B, (also known as MMP-2 and MMP-9 respectively) are directly involved in the pathogenesis of coronary thrombosis, atherosclerosis, myocardial infarction, and heart failure [156]. MMP-2 also contributes to inflammation, growth, cell migration and differentiation [157]. These enzymes are involved in the cleavage of proteins from the networks of elastin and collagen. Moreover, it has been described that the MMP-2 can digest the components of contractile machinery such as troponin I or myosin 1 light chain [156].

During ischaemia, it has been demonstrated that the proteolytic activity of MMPs in myocardium generally tends to increase. Moreover, the imbalance of MMPs and TIMPs may contribute to acute myocardial ischaemia-reperfusion injury [158]. Conversely, MMP-2 is associated with myocardial dysfunction, contributing to the development of cardiomyopathies [159,160,161,162,163]. The

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occurrence of an imbalanced regulation of NT-truncated intracellular isoform of MMP-2 (NT-MMP-2), as a consequence of oxidative stress and mitochondrial dysfunction, seems to be involved in myocardial injury [164]. Due to the tight connection of NT-MMP with the mitochondria [165, 166], it is likely that pathophysiological events, subsequent to hyperglycaemia, may underlie a cascade of events mediated by oxidative stress and mitochondrial dysfunction. These events lead to altered modulation of MMPs and, at the end stage, to failing myocardium such as that which occurs in the hearts of diabetic patients.

Recently, Gliozzi et al. found that an exaggerated NADPH oxidase-dependent production of superoxide anion (O_2^-), which occurs in hyperglycaemic rats, gradually impairs myocardial structure and function [167]. In particular, it has been demonstrated that during the early phase of cardiac injury caused by chronic hyperglycaemia, the reduced ejection fraction (EF) and fractional shortening (FS) were associated with a change in the outer mitochondrial membrane. This mechanism is characterised by O_2^--induced overexpression of the mitochondrial translocator protein (TSPO), a protein located at the level of outer mitochondrial membrane [166,168]. The major target of TSPO is the modulation of mitochondrial ion channels in response to oxidative stress [168]. Consequently, altered TSPO expression in the hearts of diabetic patients represents the link between the altered modulation of Ca^{2+} traffic across the mitochondrial membrane and the imbalanced cytosolic Ca^{2+} concentration which is found in dysfunctional cardiomyocytes of diabetic patients [169]. These mechanisms likely play a crucial role in characterizing the myocardial dysfunction found in patients undergoing diabetic cardiomyopathy.

Indeed, Gliozzi et al. also demonstrated that TSPO contributes to determine ER stress and the translocation of the intracellular NT-MMP-2 into mitochondria [167]. This is likely caused by the imbalance of mitochondrial Ca^{2+} uptake, thus causing an impairment of the function of these organelles. In this context, it has been also demonstrated that the driving cause of ventricular dysfunction in the heart of diabetic patients depends on NT-MMP-2 intracellular localization which

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affects mitochondria, rather than on the enhanced degradation of ECM. These effects may be counteracted by natural antioxidants and natural SGLT2 inhibitors as shown below.

2.4 cGMO/NO pathway in HF

Evidence exists that when functioning normally, nitric oxide synthase (NOS), nitric oxide (NO), and soluble guanylate cyclase (sGC) all oppose pathological remodelling, at least in part through important roles in the cardiac myocyte [170]. A large body of work supports that NO, the activator of sGC, also becomes dysregulated in HF and in conditions predisposing to HF [171]. When NOS expression and activity is reduced, cardiovascular tissue release of NO can be directly reduced by ROS [172]. In humans, the reduced endothelial NO (eNOS) release predisposes to HF. These reduced eNOS release conditions include ageing, diabetes, and obesity [173]. In fact, based on this human data, a recent model postulates that reduced NO-induced cyclic guanosine monophosphate (cGMP) generation leads to HFpEF [174]. Data from animal studies have also shown that eNOS becomes uncoupled not only in hypertension [175] but also in the failing heart [176]. This promotes production of toxic ROS species, rather than NO. In addition, GCs can be modified by several HF risk factors and conditions of oxidative damage, making it unable to generate cGMP in response to NO. Intracellular localization sGC in cardiac cells is also influenced by oxidation [176]. However, the failing heart also exhibits changes in cGMP catalysis through upregulation of cGMP-specific phosphodiesterases (PDEs). PDEs, part of an 11-member family (PDE1 to PDE11), catabolize cAMP, cGMP, or both; depending on the specific PDE [177]. Recently, PDE5 and PDE9, both cGMP-specific PDEs, have been studied. According to samples taken from end stage failing myocardium, PDE5 expression increases in the failing human left ventricle (LV) [178]. Pharmacologic and genetic manipulation studies in mice have also confirmed the effectiveness of PDE5 inhibition in opposing cardiac remodelling and the pro-remodelling role of the enzyme in the cardiac myocyte [178]. More recently, the cGMP-selective PDE9 was observed to increase in the failing human LV both in HFrEF and in HFpEF, as well as in the LV of mice

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subjected to experimental transverse aortic constriction (TAC) [179]. Genetic deletion or pharmacologic inhibition of PDE9 improves LV remodelling after thoracic or abdominal aortic constriction as well as in response to isoproterenol (ISO) infusion [180,181].

The collected observations above describe a general model in which alterations in cGMP-generating and anti-cGMP-generating components lead to net reduction of myocardial cGMP and subsequent cardiac remodelling and failure. Though difficult to prove causality in humans, studies on myocardial tissue of HFpEF patients have demonstrated reduced cGMP concentration, compared with not-failing tissue from patients with aortic stenosis or with HFrEF tissue [182]. Reduced myocardial cGMP has also been observed in a rat model of HFpEF [183]. Moreover, these differences correspond to reduced kinase activity of the cGMP- dependent protein kinase, as well as negatively correlating with passive stiffness of isolated cardiac myocytes [182].

In the presence of PDE5 and PDE9 inhibition, numerous animal studies also identify a net increase in cGMP [180,181]. Thus, the animal studies of cGMP regulating molecules, as well as direct observations in humans with HF, support the rationale behind pharmacological as well as nutraceutical strategies to augment intracellular cGMP in patients with HF.

2.5 HF and sodium-glucose cotransporters

In large-scale clinical trials of patients with type 2 diabetes mellitus (T2DM) and either established cardiovascular disease or multiple cardiovascular risk factors, the cardio-renal benefits demonstrated by the sodium–glucose cotransporter (SGLT)2 inhibitors were unprecedented [184]. Many of the potential benefits of regulating SGLT2 in both diabetic and non-diabetic patients with HF have been attributed to slowing f the atherothrombotic processes which accompanies T2DM and the improvement of cardio-renal consequences of T2DM which lead to failing myocardium. In addition, recent evidence suggests that SGLT2 modulation is accompanied by other effects which may be of benefit in managing HF.

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In particular, it has been proposed that SGLT2 inhibitors may improve and/or optimise cardiac energy metabolism [184]. By improving myocardial energetics and substrate efficiency, these agents may improve cardiac efficiency and cardiac output [185]. It is well established that in the presence of T2DM and/or HF, the metabolic flexibility of the heart, as it relates to substrate utilisation, is impaired. Accordingly, an over-reliance on non-esterified fatty acids (NEFAs), as a substrate for ATP generation, may result in a build-up of free fatty intermediates that may in turn promote lipotoxicity, impair SR Ca^{2+} uptake, and promote the development of diastolic dysfunction [186].

SGLT2 inhibitors, known to slightly increase the production of the ketone body β-hydroxybutyrate (βOHB), may offer an alternative myocardial fuel source which is more cost effective in those with T2DM [187]. Elevated ketone levels have been suggested to arise from an effort to raise glucagon levels, possibly through a reduction in ketone body excretion via the kidneys [188]. The underlying concept is that ketone body βOHB is a ‘super fuel’, oxidised by the heart in preference to NEFAs and glucose, and that ketones not only improve cardiac function in the failing heart, but also increase mechanical efficiency [188]. Although the cogent data to support this thesis are scarce, this is an interesting postulate. Some support, however, has been provided by preliminary studies carried out in pigs following myocardial infarction, which demonstrate that empagliflozin increases myocardial ketone consumption while reducing cardiac glucose consumption and lactate production [189]. Another hypothesis is that SGLT2 inhibitor-induced increases in βOHB levels may inhibit the histone deacetylase and prevent prohypertrophic transcription pathways [190]. It is also possible that a decrease in βOHB oxidation results in decreased acetyl-CoA, a derivative of ketone oxidation, thereby increasing glucose-derived pyruvate oxidation (i.e. improving myocardial glucose metabolism). Improvement in mitochondrial energy production may be due to a decrease in acetyl-CoA supply which may also decrease harmful hyperacetylation of mitochondrial enzymes, thereby improving mitochondrial energy production [190]. Using an elegant untargeted metabolomics approach, inhibition of SGLT2 was suggested to promote degradation of branched-

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chain amino acid (BCAA), thus providing an alternative fuel source for the failing myocardium. Degradation of BCAA is known to be impaired in HF patients and may contribute to aberrant myocardial bioenergetics [191]. Although the findings are intriguing, it must be noted that we currently lack definitive evidence that links myocardial energetics to the beneficial effects of SGLT2 inhibition. An emerging and promising hypothesis is that SGLT2 inhibition and direct effects on Na^+/H^+ exchange may directly inhibit the Na^+/H^+ exchanger (NHE) 1 isoform in the myocardium.

It has been demonstrated in experimental models of HF that NHE1 activation results in increased cytosolic Na and $Ca2^+$ [192]. Recently, Baartscheer et al. showed that the SGLT2 inhibitor empagliflozin inhibited cardiomyocyte NHE, thereby reducing cytoplasmic Na^+ and $Ca2^+$ levels while increasing mitochondrial $Ca2^+$ levels [193].

The mechanism by which these effects occur on cardiomyocyte NHE remains elusive, as SGLT2 receptors are not expressed in the heart. Notably, it has been postulated that SGLT2 inhibitors promote natriuresis by downregulating the activity of NHE3 in the proximal tubule [194]. In HF patients, the expression of NHE3 is increased and known to mediate reuptake of tubular Na^+. As an additional mechanism, the inhibitory effects on NHE3 may serve to reduce cardiac failure and restore whole-body Na^+ homeostasis [195]. Hence, a common cardio–renal mechanism through which these agents prevent and/or treat HF may be through inhibition of NHE1 and NHE3 [195].

Cardiac structural remodelling is universally involved in this mechanism, due to deposition of ECM proteins laid down by cardiac fibroblasts which impede ventricular compliance and accelerate development of HF [196]. Recent experimental of postmyocardial infarction demonstrate that dapagliflozin shows marked cardiac antifibrotic effects by suppressing collagen synthesis through increased activation of M2 macrophages and inhibition of myofibroblast differentiation [197]. Other *in vitro* studies have demonstrated that empagliflozin significantly attenuates Tumor Necrosis Factor- α (TGF-β1)-induced fibroblast activation and reduces cell-mediated ECM remodelling as

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measured by the collagen fibre alignment index [198]. Moreover, it has been demonstrated that empagliflozin suppressed expression of pro-fibrotic markers such as type I collagen, α-smooth muscle actin, connective tissue growth factor, and MMP-2 [199]. Therefore, it has been postulated that SGLT2 inhibition may have direct effect on one of the most important factors of HF: cardiac fibroblast phenotype and function. These effects indicate potential for using nutraceutical supplementation with natural SGLT2 inhibitors such as phlorizin-rich apple extract.

2.6 Impairment and senescence of cardiac stem cells in HF

The primary etiology of HF are myocardial infarction and ischaemic heart disease in general [200,201,203]. Furthermore, where HF is of non-ischaemic origin, the primary issue is the lack of the myocardium to undergo a robust cardiomyocyte replacement in cases of structural as well as "functional" cardiomyopathies [203]. Regenerative medicine aims to find a therapy that is effective and widely available to refresh the contractile muscle cells lost and/or rendered permanently dysfunctional as a consequence of the primary injury [203]. Unfortunately, the scepticism regarding the intrinsic endogenous regenerative capacity of the adult mammalian heart, has produced contradictory approaches in myocardial repair/regeneration. This scepticism can only be overcome if hard clean and clear scientific data are obtained, thereby excluding the need for interpretations and opinions. It is unlikely that any clinical repair or regeneration protocol will be ever able to answer the question regarding the feasibility of functionally regenerating the failing human heart [203]. The mammalian heart, including the human heart, contains a pool of resident tissue-specific cardiac stem/progenitor cells, named endogenous cardiac stem cells [204,205]. The eCSCs population, originally, have been identified through the expression of specific membrane markers, i.e. the stem cell factor (SCF) receptor kinase c-kit [206], Sca-1 [207], and MDR-1 [208]. *In vitro* and *in vivo* experiments have clearly shown that CSCs have all the characteristics to be considered a tissue-specific stem cell: they are clonogenic, self-renewing, and multipotent. Differentiation of the main myocardial cell is possible *in vivo* and *in vitro* [204]. Several studies reproduced the findings

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that while W locus mouse mutants (W/Wv) exhibit c-kit cell dysfunction [209,210], c-kit signalling *in vitro* promotes growth, survival, and proliferation of human CPCs [211]. Indeed, W/Wv mice display impaired cardiac recovery after infarction [212], diminished cardiac function with advanced age [124], and compromised c-kit cell differentiation into cardiomyocytes [213,214]. Bone marrow c-Kit^{POS} cells from W locus mutants or cells with silenced c-kit *in vitro,* showed blunted reparative responses to myocardial injury [210]. Moreover, being c-kit gene deletion incompatible with life the deletion of the c-kit gene, occurring in homozygous W-mutated mice, causes murine premature death [214]. Interestingly, c-kit-defective adult hearts appear to develop normally during embryonic life [215], while adult c-kit Cre-KI mice have a clear defect in their regeneration potential in myocardial infarction model [216]. Therefore, it seems that c-kit plays a divergent role in cardiac regeneration and heart formation/development, which suggests that the molecular program involved in cardiac regeneration does not resemble cardiac generation [217]. Finally, the role of c-kit was evaluated in several models of cardiac pathology such as doxorubicin (DOXO)-induced cardiomyopathy [218], chronic heart failure [219,220], and aging cardiomyopathy [221,222]. Huang et al developed a “paediatric” model of DOXO-induced cardiotoxicity where juvenile mice exposed to DOXO, did not induce acute cardiotoxicity [223]. These mice have no cardiac abnormalities as adults. However, the hearts have abnormal vasculature and a reduced number of c-kit^{pos} cardiac cells, which correlated with an increased sensitivity to physiological and pathological stimuli [223]. Subsequently, it was demonstrated that DOXO-induced cardiomyopathy is caused by a depletion of the functional c-kit^{pos} CSC pool which can be rescued by restoring their function via nutraceutical supplementation [224].

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3. Candidates for nutraceutical supplementation in the failing myocardium according to aforementioned emerging mechanisms

3.1 CoQ10

Coenzyme Q10 (CoQ10) also defined ubiquinone (in the oxidized form) or ubiquinol (in the reduced form), plays a key role in the functioning of the METC being responsible for the transfer of electrons from complex I and II to complex III thus promoting ATP generation [225] (Figure 1).

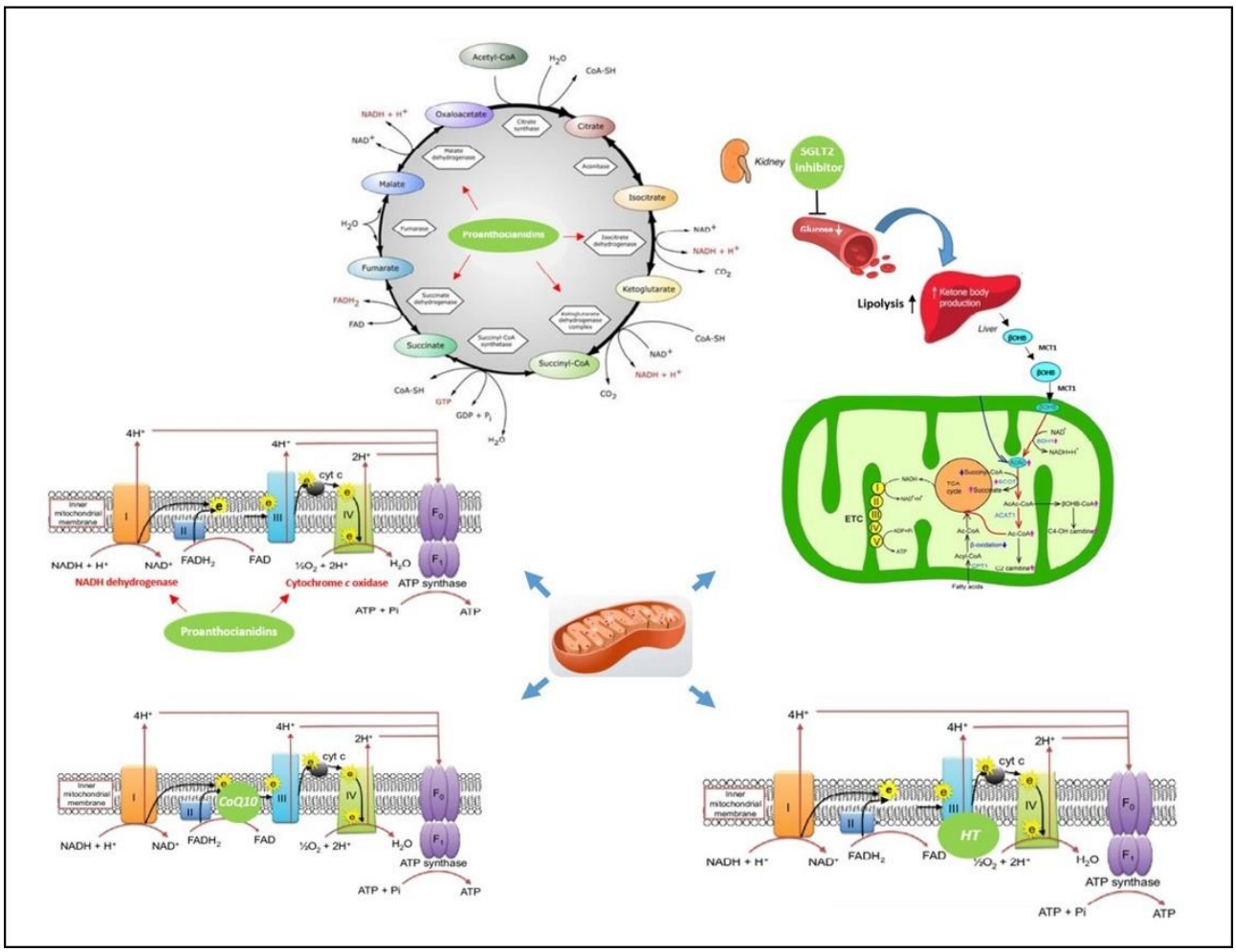

Figure 1. Effects of nutraceuticals on mitochondrial function in heart diseases

CoQ10 plays a critical role in ATP generation by accepting electrons from complexes I and II and transporting them to complex III of mitochondrial electron transport chain. Moreover, CoQ10 is involved in the protons' transfer in the inner mitochondrial membrane, called proton motive Q-cycle, leading to free movement of protons through the internal mitochondrial membrane; SGLT2

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inhibitors increase the amount of ketone bodies; Proanthocyanidins significantly increased the activities of mitochondrial enzymes (isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase and α-ketoglutarate dehydrogenase) and respiratory chain enzymes (NADH dehydrogenase and cytochrome c oxidase); HT is able to improve the integrity of complex-III of mitochondrial electron transport chain.

Moreover, CoQ10 is involved in the so called proton motive Q-cycle which enables the passage of protons across the internal mitochondrial membrane [226]. The highly lipophilic molecular structure is related to vitamin K, where “Q” connotates quinone, and “10” the 10-isoprene group [227]. CoQ10 is ubiquitous in most mammalian tissues, with particularly high levels in organs, such as the heart, with the highest rate of metabolism [227]. The higher concentration of CoQ10, compared to other carriers, balances its slower cyclic oxidation/reduction rate [228]. Therefore, a CoQ10 deficiency in the cardiac mitochondria lead to a dysfunction of the mitochondrial respiration, conversely, CoQ10 supplement could improve mitochondrial function [18]. Preclinical data suggest that CoQ10 has important anti-inflammatory properties and is able to protect endothelial function from damage [19]. In fact, CoQ10 also regulates eNOS function in different cellular membranes Thus, depletion of CoQ10 can promote uncoupling of eNOS, making it an additional source for ROS and thereby, shifting the nitrous-redox balance towards oxidation [20]. Interesting data have demonstrated a protective role of CoQ10 in high glucose-induced endothelial progenitor cell (EPC) dysfunction [21]. In particular, the administration of CoQ10 reduces apoptosis cell death and increases mitochondrial membrane potential. In addition, CoQ10 is able to reduce ROS production enhancing eNOS/Akt activity, and upregulating HO-1 expression [21] (Figure 2).

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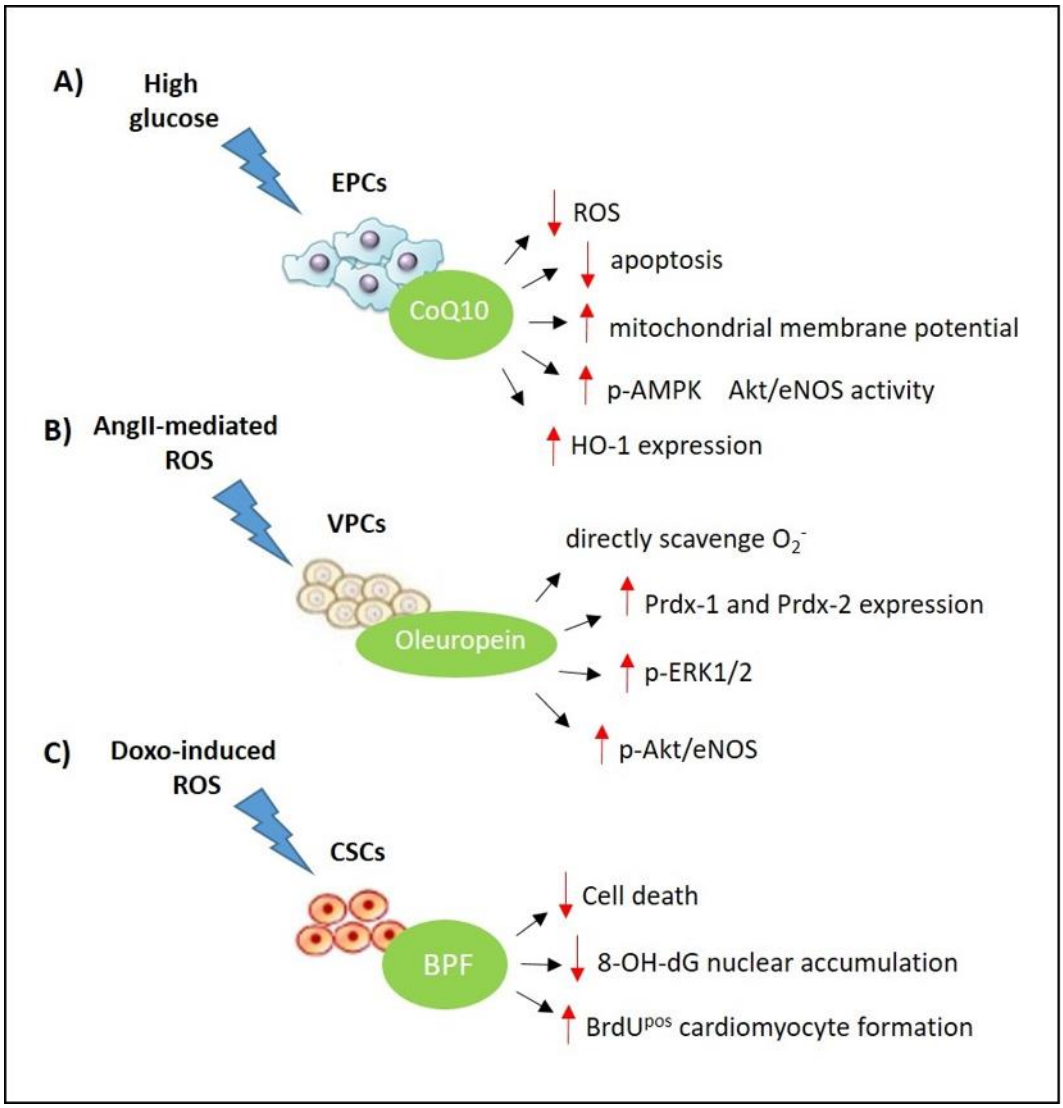

Figure 2. Effects of nutraceuticals on stem cell compartment in heart diseases

A) CoQ10 inhibits high glucose-induced EPC dysfunction and death via modulation of AMPK pathway upregulating eNOS activity and HO-1 expression; **B)** Oleuropein attenuates AngII mediated oxidative stress in VPCs though direct scavenging activity and regulating ERK1/2-Prdx and Akt/eNOS signaling pathway; **C)** BPF protects endogenous CSCs against Doxo-induced cardiotoxicity though its direct antioxidant properties and stimulating CSCs activation and differentiation in newly formed cardiomyocytes.

Physiologically, CoQ10 is ingested for about 50%, while the remaining 50% is endogenously synthesized through the mevalonate pathway which is inhibited by a statin action [22]. Indeed, in

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ISO-induced HF in rats it has been shown a severe LV dysfunction when the animals were treated with high dose of atorvastatin. Conversely, co-administration of CoQ10 with lower dose of atorvastatin ameliorates myocardial necrosis and fibrosis and the hemodynamic depression improving left ventricular (LV) dysfunction [23]. Hence, patients with cardiovascular risk and/or disease treated with statins may experience CoQ10 deficiency [18]. In patients with HF, CoQ10 deficiency is associated with the severity of disease [24]. Experimental evidence suggest that lipid peroxidation, resulting from oxidative stress, causes a reduction in CoQ10 levels [25]. At first, in a preliminary clinical study conducted in patients with HF, CoQ10 was defined as an independent predictor of mortality [26]. Instead, the data obtained from the larger CORONA study did not confirm this. According to the CORONA study results, treatment of patients with rosuvastatin did not reducef the primary endpoint of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke due to ischaemic cardiomyopathy [27], but did reduce CoQ10 concentrations. Nevertheless, the administration of rosuvastatin in patients with a low baseline of CoQ10, did not lead to a worse outcome [27]. In accordance, CoQ10 deficiency was not considered an independent prognostic factor in HF [27]. In light of this evidence, CoQ10 deficit could play a causal role in patients suffering from HF even not treated with statins.

CoQ10, as an established mitochondrial therapy, ameliorates CoQ10 biosynthesis defects through constant dosing of CoQ10 dietary supplementation [28]. However, despite the lack of firm evidence of benefit in cardiovascular diseases, CoQ10 supplementation has been studied for several decades. Though several clinical trials have shown that CoQ10 may enhance LVEF, other well-designed studies were needed to assess its effect on patient outcome [29]. Recently, in the Q-SYMBIO trial, the effects of CoQ10 were studied in 420 patients with systolic HF, showing a significant improvement in symptoms and a substantial reduction of major adverse cardiovascular events [30]. However, the study was underpowered and failed to provide enough evidence of a benefit in this population, therefore it is still unclear if CoQ10 administration benefits outcome and symptoms in HF [30]. Moreover, CoQ10 is not mentioned in the recent guidelines on the treatment of HF [19].

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3.2 Bergamot Polyphenols

Scientific evidence shown that bergamot polyphenolic fraction (BPF) improves serum lipemic profile and modulates blood pressure in patients with metabolic syndrome (MetS), a state which contribute to the development of HF [31,32,33]. Previous scientific evidence obtained with citrus fruits, shown their beneficial effects and clarify some mechanisms involved in MetS [34,35].

It has been reported that citrus peel extract, rich in pectins and flavonoids, significantly reduces cholesterol levels by modulating hepatic HMG-CoA levels [36]. Moreover, interesting data shown that bergamot juice increases the excretion of faecal sterols in rats [37,38,39].

The hypolipemic effect of BPF seems to be related to the modulatory properties of naringin and neohesperidin. Indeed, dietary hesperetin inhibits hepatic triacylglycerol (TG) accumulation, which may be associated with the reduced activity of TG synthetic enzymes, such as phosphatidate phosphohydrolase [33]. In addition, *in vitro* studies showed that naringenin and hesperetin decrease the availability of lipids for assembly of apoB-containing lipoproteins. This effect seems to be mediated by reduced activity of acyl-CoA-cholesterol acyltransferase (ACAT) [35].

In addition, BPF is rich in brutieridin and melitidin, which are 3-hydroxy-3-methylglutaryl derivatives of hesperetin and naringenin, respectively.Several studies reported that naringin, theglycoside derivative of naringenin, can downregulate the hepatic HMG-CoA reductase [31]. Thus, the strong hypocholesterolemic properties of BPF might be attributed to the activity of melitidin, brutieridin, together with naringin and other favonone glycosides.

Recently, it has been showed, that eNOS knockout mice have a cluster of cardiovascular risk factors comparable to those of MetS. These data suggest that defects in eNOS function may be involved in MetS and can induce an impaired vasodilation mediated by a clear reduction of NO levels [40].

Interesting data suggest that citrus flavonoids inhibit ROS overproduction in the vascular wall

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through antioxidant and anti-inflammatory mechanisms [39]. In particular, citrus flavonoids significantly enhance superoxide dismutase (SOD) and catalase (CAT) activities and preserve plasma vitamin E [39]. These mechanisms may restore the imbalanced endothelial function observed in patients with hyperlipemia treated with BPF [41,42]. Another described benefit of BPF is linked to its hypoglycaemic activity. It has been shown that naringenin, and other polyphenols, significantly increased AMP kinase (AMPK) activity and glucose uptake in muscle cells and liver [43]. Moreover, the hypoglycaemic activity of insulin sensitivity and glucose tolerance of naringenin has been described in animal models of MetS [44].

Based on these evidence, BPF supplementation could be considered a new phytotherapeutic approach for the management of pre-diabetic states in patients with MetS due to its hypocholesterolemic and hypolipidemic effects improving NO-dependent vasoreactivity and reducing blood glucose.

BPF may have lipid-lowering properties, synergizing with statins thus enhancing the antioxidant activity. In particular, it is reported that statins have cholesterol-independent pleiotropic effects such as the specific antioxidant activity of NADPH oxidase inhibition [7], antioxidant enzymes (SOD1, SOD3, and GPx) induction [7,45], eNOS uncoupling prevention, and eNOS increase [45]; but the aforementioned properties are often hindered by the side effects of statins; however, this condition may be overcome by the use of a therapy combined with antioxidants [7]. Indeed, it has been demonstrated that BPF supplementation enhances the antioxidant properties of rosuvastatin and significantly reduces oxidative stress in circulating polymorphonucleates (PMC) of patients with mixed hyperlipidaemia [41]. Additionally, malondialdehyde (MDA) levels in PMC decrease when BPF is added to rosuvastatin [41].

Moreover, treatment with rosuvastatin or BPF alone downregulated the expression of lectin-type oxidized LDL receptor 1 (LOX-1) and phospho protein kinase B (PKB). Such effects were further rised in patients taking both compounds [41].

As LOX-1 and phospho PKB expression are relevant biomarkers of vascular cell viability it is

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conceivable that the co-treatment with statins and BPF lead toan additional vasoprotective effect in patients with cardiometabolic risk [41].

Recent data published by Carresi et al. have also shown that BPF treatment leads to relevant benefits in doxorubicin (DOXO)-induced cardiac damage in rats, preserving LV contractility and attenuating pathologic myocardial remodelling [224]. Moreover, they have shown that BPF strongly prevents the induction of ROS, the excessive expression of pro-autophagic mediators, and myocyte apoptotic cell death. Intriguingly, for the first time, the authorshave demonstrated that BPF is also able to attenuate attrition in endogenous cardiac stem cells (eCSCs), thereby improving the number of resident c-Kit^{pos}CD45^{neg}CD31^{neg} eCSCs, inhibiting 8-hydroxy-2' -deoxyguanosine (8-OHdG) nuclear accumulation, and promoting cardiomyocyte replenishment with an increasing number of small, newly formed BrdU^{pos} myocytes after DOXO administration [224] (Figure 2).

Indeed, previous experimental evidence has been collected demonstrating that the use of DOXO impairs endogenous resident cardiac progenitors; this was identified as an inherent feature of DOXO cardiotoxicity. In addition, it has been reported that infusion of isolated human CSCs in DOXO-induced heart disease in different experimental models is able to repair the damage and ameliorate cardiac function [46].

Therefore, the main findings from the Carresi et al. study revealed the highly beneficial cardioprotective effects of BPF against DOXO-induced cardiomyopathy through its direct scavenging and antioxidant properties. The antioxidant effect of BPF enables it to interfere with production of DOXO-induced free radicals, excessive autophagy activation, and apoptosis; thus preventing the compensatory mechanisms and pathological changes that lead to the development of cardiomyopathy [224].

Overall, these data show a widely beneficial effect highlighted by BPF not only on cardiomyocytes but also likely on the compartment of resident eCSCs; these may represent only part of the cardioprotective effects of bergamot-derived polyphenols.

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BPF has clear protective effects on the heart and is likely involved in the maintenance of functional eCSCs. Even if some of the beneficial effects of BPF can be clearly attributed to its direct scavenging and antioxidant activities the protective molecular mechanism involved in the maintenance of resident eCSCs is not yet fully understood.

Further work is required to clarify the specific molecular and genetic mechanisms underlying the onset of cardiomyopathy induced by DOXO and to confirm the findings about the role of BPF in cardioprotection.

3.3 Olea Europea L. Extract

Olea europaea leaf extract (OLEA) is responsible for the major part of the beneficial effects that the Mediterranean diet has on human health [229,230,231,232,233]. In the past, the richness of monounsaturated fatty acids (MUFA), was considered the major healthful characteristic of OLEA. Following other observation made on aliments rich in MUFA, such as rapeseeds, soybean, and sunflower, it is quite clear that none of those aliments are comparable with with OLEA as healthful food [234,235]. The compounds found in OLEA could maintain their biological activity when consumed in crude form. More than 200 ‘minor components’ have been retrieved in the unsaponifiable fraction of olive oil; representing about 2% of the total weight and including a number of heterogeneous compounds non-chemically related to fatty acids [236,237].

In the last years great attention has been focused on the nutraceutical properties of OLEA compounds providing antioxidant activity. The most abundant antioxidants in OLEA are lipophilic and hydrophilic phenols [238], normally synthetized by the plants to react against pathogens and/or insects [239,240].

OLEA nutraceutical properties have been attributed mainly to oleuropein (OL) and its derivatives; the hydroxytyrosol (HT) and *p*-hydroxyphenyl ethanol or tyrosol [241,242]. These compounds are released from the olive drupes into OLEA during the phase of the extraction process. In particular, oleuropein is abundant in unprocessed olive leaves and drupes, while higher concentration of HT

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could be found in the drupes and in olive oil, following the chemical and enzymatic reactions occurring in the plant during maturation of the fruit [47]. In addition, many factors related to the agronomy, such as cultivar, ripening stage, geographic origin of olive drupes, and olive trees irrigation; as well as oil extraction conditions during crushing, malaxation and OLEA separation may influence their final concentration [48].

OL and HT represent the most investigated antioxidant molecules for their biological and pharmacological properties [49]. A wide variety of beneficial effects, related to their antioxidant activity , have been shown, in several preclinical models of diseases [47].

The antioxidant activity of OL and HT *in vivo* is related probably to their high bioavailability [50,51]. OL and HT are free radical scavengers, radical chain-breakers, anti-oxygen radicals, and metal chelators [52]. They could break peroxidative chain reactions and scavenge the peroxyl radicals with their catecholic structure; producing stable structures [53]. *In vitro* studies have shown a decrease in ROS production after treatment with OL or HT, suggesting a chelating action on such metal derived from copper ion-induced low-density lipoproteinoxidation (oxLDL) [54]. Moreover, other data suggest cardioprotective effects of these molecules. In fact, in a preclinical model of myocardial injury, the antio-oxidant effects of OL was observed [55]. In particular, the levels of creatine kinase and oxidize glutathione were significantly reduced after the treatment with oleuropein, suggesting a cardioprotective effect in the acute events following coronary occlusion [155]. Furthermore, it has been observed that oleuropein could prevent cardiomyopathy in rats treated with doxorubicin [56]. In addition, Granados et al have reported that HT attenuated DOXO-associated chronic cardiac toxicity in rats with breast cancer; thus ameliorating mitochondrial dysfunction [57]. In particular, HT treatment significantly prevents mitochondrial swelling and vacuolization improving the integrity of complex-III of the METC after DOXO treatment [57] (Figure 1). Treatment with OL reduced also the infarct size in normal and hypercholesterolemic rabbits [58]. Additionally, OL protection of reperfused myocardium was associated with a reduction in total cholesterol and triglyceride levels [58]. HT reduced the expression of proteins related to

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ageing as well as the infarct size and apoptosis cardiomyocytes [59]. A reduced infarct size, with concomitant improvement of the myocardial function was shown in tyrosol-treated rats compared to non-treated animals [60]. In particular, oleuropein was able to inhibit the myocardial infarction size and to reduce the levels of CK-MB and LDH in the myocardial I/R rats. Furthermore, it has been reported that oleuropein might be cardioprotective, acting on ERK pathway suppressing the induction of p53, p-MEK, and p-ERK protein expression in myocardial I/R rats [61]. Oleuropein attenuated cisplatin-induced acute renal injury through inhibition of p53 and ERK signalling in mice [62].

The cardioprotective effects of OLEA and HT, through endoplasmic reticulum (ER) stress prevention, have been demonstred [63]. The administration of HT prevents ER stress induced apoptosis by inhibiting mRNA and protein expression of GRP78 and CHOP in hypoxia-induced H9c2 cells. Moreover, pre-treatment with OLEA prevents inflammation and infarction size, improving EF and FS in ISO-induced myocardial infarction in rats [63].

The the protective effects of oleuropein on stem cells has been also reported. Interestingly, oleuropein prevents oxidative stress directly scavenging O_2^- in AngII-mediated human vascular progenitor cell (VPC) depletion. Furthermore, it was shown that OL exerts its protective effects via upregulation of ERK1/2-Prdx1 and 2 and Akt/eNOS signalling pathway [64] (Figure 2).

3.4 Apple-derived natural $SGLT_2$-inhibitors

Phlorizin is the main phenolic-glucoside in apple trees. It is present in roots, bark, shoots, and leaves [243]. It is a naturally competitive inhibitor of SGLT2, providing the first insights into the potential efficacy of this compound [244].

Many effects of phlorizin-rich extract contribute to its potential use in treatments to ameliorate diabetes and other metabolic disorders. In fact, it has been reported that apple juice and apple extracts, contain a total phenolic concentration of 11%-36% phlorizin which could inhibit oxLDL levels [65]. Additionally, the aglycon of phlorizin, phloretin, produces endothelium-independent

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relaxation of isolated coronary artery rings [66,67]. In 1987, Rossetti et al were the first to demonstrate glucose-induced desensitization in diabetes, now known as glucose toxicity [68]. The authors reported that phlorizin was able to improve insulin sensitivity by lowering blood sugar. Moreover, it is likely that phlorizin normalized insulin sensitivity animals with diabetes, by using partially pancreatectomized rats pre-treated with phlorizin to block renal glucose resorption [69,70]. Subsequent studies confirmed that the normalization of blood glucose in diabetic rats occurred without changing the expression or membrane concentration of adipose cell glucose transporters [71]. Numerous other studies on insulin resistance have used phlorizin as a way to control the effects of hyperglycaemia *per se* on insulin action [67]. In fact the rise in blood sugar following ingestion of a glucose solution was blunted after orally administration of phlorizin to mice [72]. Based on these observations, a phlorizin derivative was developed: the 3-(Benzo[b]furan-5-yl)-2′,6′-dihydroxy-4′-methylpropiophenone-2′-O-(6-O-methoxycarbonyl)-β-d-glucopyranoside (T-1095). Administered orally, this derivative inhibits the renal sodium–glucose symporter. T1095 acutely increases urinary glucose excretion and lowers blood glucose levels in mouse and rat models of T1DM and T2DM. Moreover, chronic treatment with T1095 reduces glucose and glycohemoglobin levels, lowers insulin and triglyceride levels, normalizes body and muscle Glucose transporter type 4 (GLUT 4) content and decreases hepatic glucose production [73]. Numerous renal effects of T-1095 in animal models of diabetes, including delays in the development of microalbuminuria, normalization of renal GLUT 2 levels, restoration of normal kidney weight, and epithelial vacuolation, were reported [74]. Nevertheless, the specific mechanism underlying the protective effects of SGLT2 inhibitors on cardiovascular diseases is to be clarified, since SGLT2 is not expressed in cardiomyocytes [75]. Potential cardiovascular protective effects of SGLT2 inhibitors, comparable to the anti-heart failure drugs, currently used, were recently described [76]. Pre-clinical data suggest that the cardioprotective effects of SGLT2 inhibitors, involve the mitochondrial function, modulating the environment outside the myocardium or through a direct effect in the heart [77]. Indeed, the administration of SGLT2 inhibitors in the damaged

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heart induce the production and release of ketone bodies which lead to oxidation of the mitochondrial coenzyme Q couple promoting ATP hydrolysis and energy resource [78] (Figure 1).

3.5 Grape seed extract

Proanthocyanidins (PCs) are naturally occurring polyphenolic compounds, widely available in a variety of fruits, vegetables, nuts, seeds, flowers, and bark [245]. Grape seed PCs, a combination of biologically active polyphenolic flavonoids including oligomeric PCs, have demonstrated to exert a novel spectrum of biological, pharmacological, therapeutic, and chemoprotective properties against oxygen free radicals and oxidative stress [245]. Interestingly, grape seed PCs have shown cardioprotective effects through the modulation of mitochondrial and lysosomal function. In particular, co-treatment with PCs significantly increase the activities of mitochondrial and respiratory chain enzymes and reduce the activities of lysosomal enzymes in the heart tissues of ISO-treated rats [79] (Figure 1).

Recently, the cardioprotective ability of an innovative formulation of grape seed proanthocyanidin extract (GSPE), obtained from HEAD Italia Srl, laboratories was evaluated. GSPE is a natural, standardized, water–ethanol extract from seeds of the Calabrian red grape. High-pressure liquid chromatography (HPLC) analyses in conjunction with gas chromatography–mass spectrometry (GC–MS) demonstrated that GSPE contains approximately 75–80% oligomeric PCs and 3–5% monomeric PCs [80]. In an *in vitro* model, GSPE exhibited dramatic concentration-dependent scavenging ability towards biochemically generated superoxide anion, hydroxyl, and peroxyl radicals; providing significantly better scavenging compared to Vitamins C and E [81].

Additionally, GSPE exhibited excellent dose-dependent protective ability against: 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced hepatic peroxidation, brain lipid peroxidation, DNA fragmentation; and peritoneal macrophage activation in mice [82]. In a primary culture of human oral keratinocytes, GSPE also demonstrated significant protection against smokeless tobacco-induced oxidative stress, DNA damage and apoptotic cell death [83]. Moreover, while

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enhancing the growth and viability of normal cells, GSPE exerted significant cytotoxicity towards human breast, lung, and gastric adenocarcinoma cells [84,85]. By modulating apoptotic regulatory genes bcl-2, c-myc, and p53, GSPE also exhibited significant protection against chemotherapeutic drug-induced cytotoxicity in normal human liver cells [83]. GSPE provided excellent *in vivo* protection against structurally diverse drug- and chemical-induced multiorgan toxicity [86], protecting against acetaminophen-induced hepatotoxicity by dramatically enhancing the expression of the bcl-XL gene in the liver tissue [87]. These studies demonstrate that GSPE, a potent scavenger of free radicals, is bioavailable and provides significant protection to multiple target organs against structurally diverse drug- and chemical-induced toxic manifestation [88].

Using a well-established technique, cardiomyocytes were obtained in order to examine apoptosis [89]. The TUNEL assay demonstrated that GSPE significantly reduced the incidence of cardiomyocyte apoptosis. The number of apoptotic cells in the ischaemic–reperfused myocardium was significantly greater (23%) compared to that of the control hearts, in which GSPE treatment significantly reduced the number of apoptotic cells (by approximately 69%) compared to the ischaemic–reperfused hearts. Thirty minutes of ischaemia followed by two hours of reperfusion significantly enhanced JNK-1 and c-JUN protein levels, which were dramatically reduced by pretreatment with GSPE. Specifically, the JNK-1 protein level was reduced by approximately 57%, while the c-JUN protein level was reduced by approximately 54% [89]. Previous studies have demonstrated that JNKs are activated during the reperfusion of ischaemic myocardium [90]. At least two members of the JNK family, JNK-1 (46 kDa) and JNK-2 (55 kDa), can phosphorylate c-JUN on specific N-terminal serine residues [91]. Several other observations have documented that JNK-1 is activated by ischaemia–reperfusion. Ionizing radiation and most of the DNA-damaging agents that activate JNK can also generate free radicals [92]. Additionally, JNK activates the tumour suppressor p53 gene [93], a pro-apoptotic transcription factor that suppresses the anti-death gene bcl-2 and enhances Bax induction [94]. JNK can also antagonize the function of the anti-apoptotic protection of bcl-2 through phosphorylation [95]. Thus, this is consistent with earlier

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reports [89], and further suggests that GSPE can reduce cardiomyocyte apoptosis through the inhibition of ischaemia–reperfusion-induced activation of pro-apoptotic genes JNK-1 and c-JUN.

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