OPEN ACCESS Freely available online

ISSN: 2329-6925
Journal of Vascular Medicine & Surgery

Review Article

Metabolomic Regulation by Oxidative Stress in Cardiac Diseases: An Overview on the State of the Heart

Alan Goncalves Amaral[1], Hernandes F. Carvalho[2,3], Aline Mara dos Santos[2,3*]

[1]Department of Analytical Chemistry, Institute of Chemistry, State University of Campinas, Campinas, Brazil; [2]Department of Structural and Functional Biology, Institute of Biology, State University of Campinas, Campinas, Brazil; [3]National Institute of Science and Technology on Photonics Applied to Cell Biology (INFABIC), State University of Campinas, Campinas, Brazil

ABSTRACT

Associations of oxidative stress with Cardiovascular Diseases (CVDs) are well established; however, the mechanism underlying cardiac damage caused by Reactive Oxygen Species (ROS) is not fully understood. ROS are highly reactive chemical molecules capable of damaging fundamental cellular molecules, including DNA, proteins, and lipids, leading to metabolic alterations, ultimately resulting in cardiomyocyte dysfunction and heart diseases. This review will discuss the current state of metabolomics technologies, the pathological implications of oxidative stress in cardiac tissue, and the metabolic switches in the glucose, lipids, purine and pyrimidines, and glutathione metabolism orchestrated by oxidative stress and their implications on cardiac diseases.

Keywords: Cardiovascular diseases; Oxidative stress; Metabolomics

INTRODUCTION

Oxidative stress is a state of imbalance between the production of reactive oxygen species and the endogenous antioxidant defense mechanisms, resulting in excessive ROS production, which is associated with the pathogenesis of multiple cardiovascular diseases [1-6]. ROS are highly reactive compounds containing oxygen, including OH (hydroxyl), O_2^- (superoxide), $ONOO^-$ (peroxynitrite), and non-radicals such as H_2O_2 (hydrogen peroxide). Physiologically, antioxidant defenses are capable of controlling the levels of ROS by the activity of the antioxidant enzymes, including glutathione peroxidase, superoxide dismutase, and catalase. Conversely, an increase in ROS production that overcomes the antioxidant defenses leads to a series of cell damages, including DNA breaks, lipid peroxidation, and protein oxidation [7]. Indeed, the accumulation of oxidative damage is a common factor involved in CVDs progression. This review summarizes current knowledge regarding oxidative stress and its pathological actions on the heart, highlighting cardiomyocyte metabolic alterations and their implications for overall cell metabolism. Specifically, the function of the endogenous antioxidant defense mechanisms and the pathological alterations in the primary cellular source of ROS will be discussed and linked to the development of the most relevant myocardial-related diseases. Then, a discussion of metabolic switches orchestrated by oxidative stress in cardiac diseases will be presented in parallel with results reached by H_2O_2-induced oxidative stress in H9c2 cardiomyocytes.

METABOLOMIC TECHNOLOGIES

The need to investigate different phenomena and improve techniques requires developing new tools. In recent years, we have seen in the area of life sciences the development of genetic engineering with CRISPR [8,9], improvement of bioinformatics [10], new pharmaceutical designs [11-13], and the use of omics [14-16]. The latter is a popular tool lately where large-scale studies are carried out to explore from genes to metabolites [17,18]. For studies on metabolism, we have metabolomics, a term introduced by Oliver Fiehn in 2001 [18]. In general, metabolomics can be defined as the comparison between the metabolome of an object of study in its standard and altered state, being those diseases, the ripening stage of a fruit, different growth sites of a plant species [18,19]. Its application was

Correspondence to: Aline Mara dos Santos, Department of Structural and Functional Biology, Institute of Biology, State University of Campinas, Campinas, Brazil, E-mail: alinems@unicamp.br

Received: 30-May-2022, Manuscript No. JVMS-22-16816; **Editor assigned:** 01-Jun-2022, Pre QC No. JVMS-22-16816 (PQ); **Reviewed:** 22-Jun-2022, QC No. JVMS-22-16816; **Revised:** 29-Jun-2022, Manuscript No. JVMS-22-16816 (R); **Published:** 06-Jul-2022, DOI: 10.35248/2329-6925.22.10.456

Citation: Amaral AG, Carvalho HF, Santos AM (2022) Metabolomic Regulation by Oxidative Stress in Cardiac Diseases: A Short Overview of the State of the Heart. J Vasc Surg. 10:456.

Copyright: © 2022 Amaral AG, et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

facilitated by the development of Mass Spectrometry (MS) techniques, which is the most widely used tool.

Mass spectrometry, developed by J. J. Thomson in 1912 and improved by A. J. Dempster in 1918, is based on the ionization and fragmentation of molecules, generating a spectrum composed of the mass/charge ratio (m/z) of the fragments formed, which, when interpreted, provides information about the structures contained in a given sample [20]. Its use is frequent in metabolomics because of its high selectivity and sensitivity and the variety of commercially available equipment, allowing different couplings (e.g., GC-MS, HPLC-MS, and CE-MS), ionization sources (e.g., EI, ESI, MALDI, and DESI), and mass analyzers (e.g., quadrupole, TOF, and Orbitrap) [21-25]. These instruments will generate different information about the samples, which complement each other. Since the type of sample and its preparation influence the choice of instruments, the advantages and limitations of the techniques must be aware [17]. GC-MS is primarily restricted to volatile and semi-volatile compounds, and its main disadvantage is the inclusion of a derivatization procedure in sample preparation [26,27]. On the other hand, it has high robustness, separation efficiency, reproducibility, and credibility in identifying small metabolites [26,27]. The LC-MS allows the analysis of different molecules with different polarities by varying its mobile phase, being employed the Reverse Phase Mode Chromatography (RPLC) for the study of more apolar molecules and the Hydrophilic Interaction Chromatography (HILIC) mode for more polar molecules [28,29]. In CE-MS, we have the separation and analysis of polar ionic compounds present in an electrolyte by an electric field applied to a capillary [30]. Due to its more complex construction than the other two instruments, careful optimization of the electrophoretic parameters is essential [31]. When using samples such as histological sections and cultured cells, imaging techniques such as MALDI (Matrix-Assisted Laser Desorption Ionization), SIMS (Secondary Ion Mass Spectrometry), and DESI (Desorption Electrospray) are typically used. Since the sample is in its native state, i.e., there is barely any sample preparation, there is little or no loss of metabolites during the analysis [24]. These techniques also allow the cells to be studied individually. This sub-area of metabolomics, known as single-cell metabolomics, has been widely pursued due to its great potential [32]. Given all the advantages and possibilities, metabolomic techniques have the potential to improve the understanding of heart diseases, enabling the discovery of new biomarkers and therapeutic targets.

CELLULAR METABOLISM AND REDOX BALANCE

Redox reactions are constantly present in aerobic organisms, with their products participating in cellular metabolism. Among cellular components, mitochondria play a crucial role in the redox balance, being the primary source of ROS and antioxidants [33]. As is well known, the mitochondria are responsible for providing energy to the cell *via* oxidative phosphorylation performed in the mitochondrial electron transport chain. Among the five complexes responsible for this phenomenon, failures can occur in complexes I and III leading to electron leakage that, when reacting with O_2, can produce Reactive Oxygen Species (ROS) [34]. Instead of being used to reduce O_2 to H_2O by the enzyme cytochrome C oxidase, these electrons migrate to the cytosol and react with O_2, generating the superoxide radical [33,34]. This highly reactive compound derived from O_2 is primarily produced in mitochondria; nevertheless, other sources of $O_2\bullet-$, such as NADPH oxidase, monoamine oxidase, and xanthine Oxidoreductase, produce ROS throughout different reactions (Figure 1) [35].

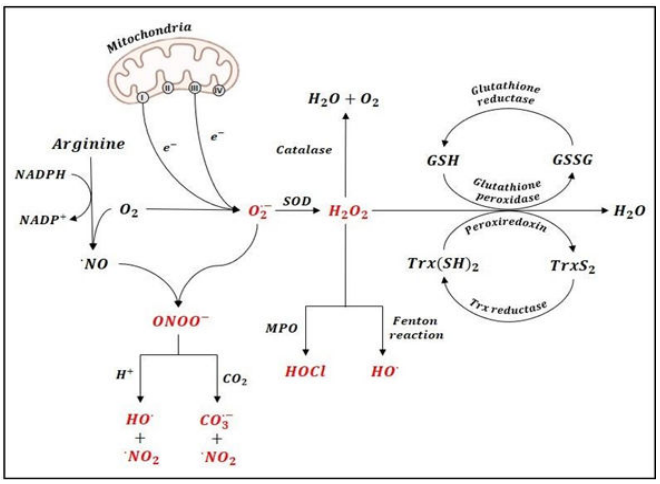

Figure 1: Redox reactions of eukaryotic cells, highlighting [red] Reactive Oxygen Species [ROS].

In response to the physiological production of ROS, eukaryotic organisms developed a range of defense mechanisms known as the antioxidant system. Such mechanisms are based on the reduction of ROS by enzymes, including Super Oxide Dismutase (SOD), catalase, and Thioredoxin (Trx), and by endogenous or non-endogenous biomolecules (e.g., glutathione, vitamins A, C, and E) [36]. Within the metabolic pathways that actively participate in the antioxidant system of organisms, glutathione metabolism plays a fundamental role. Glutathione (GSH) is the most important intracellular antioxidant. Its main action is the removal of $HO\bullet$, $ONOO-$, $CO_3\bullet-$, and HOCl. In addition, in combination with the enzymes glutathione peroxidase and glutathione reductase, GSH acts in the removal of H_2O_2. The ability to consume cell-damaging species and its regenerative power made the glutathione system a robust antioxidant defense pathway [37]. However, cells undergo oxidative stress when the antioxidant defense is insufficient and the redox balance is disrupted, leading to cellular damage. Several studies highlight the link between oxidative stress and the pathogenesis of diseases, including CVDs [5].

PATHOLOGICAL IMPLICATIONS OF OXIDATIVE STRESS IN CARDIOVASCULAR DISEASES

General aspects

Cardiovascular diseases represent the leading causes of death worldwide, according to the World Health Organization [38]. In recent decades, with the increase in the incidence of cancer,

obesity, and diabetes, in addition to the aging of the population, the risk of CVD has increased, in part due to the accumulation of oxidative damage [39]. Indeed, oxidative stress is a relevant factor in the development of cardiovascular diseases, including myocardial fibrosis and infarction, hypertrophy, and Heart Failure (HF). Thus, significant efforts have been made to elucidate the biomolecular alterations governed by oxidative stress and its consequences to CVDs progression [40-45].

The pathophysiological effects of ROS depend on their disponibility and site of production. Radical species with a shorter half-life are more unstable and toxic; conversely, the site of production and diffusion of the radical species throughout the cell and tissue impacts the surrounding molecules directly [46]. In pathological conditions, when ROS production overcomes the antioxidant defenses, ROS are able to cause oxidative modification of cellular macromolecules, such as DNA, lipids, and proteins [7]. Reactive oxygen species are a constant threat to DNA. DNA oxidation results in breaks that can generate mutations during the DNA repair, with the risk of disrupting genome function [47]. The genome instability and the mutagenic processes derived from oxidative stress are tightly linked to multiple age-related diseases, such as hypertension and diabetes [48,49]. Otherwise, the oxidation of lipids and proteins induces modifications in the sarcolemma and subcellular organelles, such as mitochondria and sarcoplasmic reticulum, impacting the production of energy and the metabolism of calcium. Such alterations may trigger cardiomyocyte dysfunction and death through apoptosis and necrosis, events linked to contractile dysfunction, impaired cardiac remodeling, hypertrophy, fibrosis, and HF [5,50,51]. Importantly, enzymatic sources for ROS, such as the Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, Xanthine Oxidoreductase, Monoamine Oxidases (MAO), Cytochrome P450 Oxidase, and mitochondria are all considered relevant sources of ROS in CVDs, causing myocardial dysfunction. In the current topic, the most relevant sources of ROS in cardiac tissue will be summarized, and the related knowledge regarding pathological actions of oxidative stress on the heart will be discussed.

NADPH oxidases

Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidases (NOXs) are a family of multisubunit enzymes comprising membrane and cytosolic components responsible for transporting electrons across biological membranes, which leads to the reduction of oxygen into $O_2\bullet-$, in a reaction that consumes NADPH as an electron donor [52]. NOX 2 and 4 represent the members that are expressed in cardiomyocytes. While NOX2 is a sarcolemmal enzyme activated by several stimuli such as angiotensin II, growth factors, endothelin-1, TNF-α, and mechanical forces [53,54]; NOX4, found in intracellular membranes, are constitutively active [53,56]. NOX2 inactivation reduces the infarct area and ameliorates the development of heart failure in animal models of myocardial infarction. However, it is unclear whether this is related to vascular NOX or the NOX located in inflammatory cells [57]. NOX2-induced superoxide generation is also involved in angiotensin II-induced cardiomyocyte hypertrophy; thereby, antioxidative therapies have been proposed to ameliorate cardiac hypertrophy by inhibiting the activity of NOX enzymes [57,58].

Furthermore, NOX activity was shown to be upregulated in patients with ischemic or dilated cardiomyopathies, which is associated with increased RAC1 activity [57,58]. NOX4 constitutively produces hydrogen peroxide rather than $O_2\bullet-$. In heart tissue, NOX4 seems to be located at the membranes of the endoplasmic reticulum, nuclear envelope, and mitochondria. Several studies have reported a protective role of NOX4 in cardiac hypertrophy and against cardiac remodeling, regulating different transcription factors, such as NRF2, HIF1α, and ATF4. Briefly, the signaling pathways modulated by NOX4-enzyme serve to (a) limit oxidative stress, mitochondrial DNA damage, and cardiomyocyte death; (b) mediate cardiac remodeling and promote angiogenesis to protect the stressed hearts; and (c) activate autophagy in response to energy stress [59].

Xanthine Oxido Reductase (XOR)

Xantina Oxido Redutase (XOR) is also an important source of ROS in human hearts. This enzyme is expressed in the dehydrogenase form, but it is converted to the oxidase form under stress conditions. Both forms oxidate xanthine to uric acid, reducing NAD+ to NADH, in the case of the dehydrogenase form, and molecular oxygen to H_2O_2 and $O_2\bullet-$, in the case of the oxidase form [60]. In failing hearts, the level of XOR expression is increased when compared with normal myocardium [61]. Recent studies showed that XO inhibition improved myocardial efficiency and reverted left ventricular remodeling in dilated cardiomyopathy and myocardial infarction [61-63]. These results suggest that free radical production by XOR may be a significant cause of myocardial-related diseases.

Mitochondrial oxidative stress

Mitochondria are the predominant source of intracellular ROS, which are produced by the mitochondrial Electron Transfer Chain (ETC) as a byproduct of electron transfer. ROS generation in mitochondria is related to the partial reduction of O_2 to $O_2\bullet-$ by complexes I and III of the electron transfer chain. $O_2\bullet-$ generated is rapidly dismutate to H_2O_2 by the Mn-dependent superoxide dismutase [64]. However, other proteins may also trigger mitochondrial oxidative stress, such as the p66shc, MAOs, and NOX4 [64, 65]. Upon stress, p66shc translocates to mitochondria and contributes to mitochondrial ROS production by oxidizing cytochrome C and stimulating hydrogen peroxide production [66].

Excessive ROS production occurs during mitochondrial dysfunction resulting in damage to mitochondrial DNA and defects in the ETC function [64]. The resulting increase in H_2O_2 levels induces cell death and left ventricular dysfunction. Mitochondrial dysfunction has been shown to play an essential role in the development and progression of HF in a murine model of myocardial infarction [45, 53,67]. Targeting catalase to mitochondria prevents HF in response to pressure overload and neurohormonal stimulation [64]. Mitochondrial ROS production is also a critical factor in many diabetes-related cardiovascular diseases [67-69]. Indeed, mice fed with a high-fat

high-sucrose diet develop mitochondrial oxidative stress and cardiac hypertrophy [69,70]. An increase in mitochondrial oxidative stress was also described in the atria of diabetic and obese patients [71]. Likewise, in an insulin-dependent diabetes mellitus model, the mitochondrial generation of ROS leads to senescence and apoptosis of cardiac progenitor cells [68]. These data indicate that increased mitochondrial ROS production plays a crucial role in the pathophysiology of cardiac diseases.

Mono Amine Oxidases (MAO)

Mono Amine Oxidases (MAO) is another source of mitochondrial ROS. These enzymes are localized in the outer mitochondrial membrane and exist as two isoforms, MAO-A and MAO-B [72]. They are members of the flavoenzymes family, responsible for oxidizing biologically active amines in mammals, and are expressed at equivalent levels in the human heart [73]. MAO uses a FAD cofactor to catalyze the oxidative deamination of dietary amines, monoamine neurotransmitters, and catecholamines hormones, including serotonin, dopamine, norepinephrine, and epinephrine. The oxidative deamination generates toxic products (NH3) and H2O2 [74]. In acute or chronic stress situations, MAO-A is a source of deleterious ROS, resulting in cardiolipin peroxidation, cardiomyocyte death, and ventricular dysfunction [75-77]. Likewise, global deletion of MAO-B protects against oxidative stress, left ventricular remodeling, and prevents cardiac failure in a mouse model of congestive heart failure induced by transverse aortic constriction [78]. The cardiac protection of MAO-B depletion was confirmed by the generation of cardiac-specific MAO-B knockout mouse submitted to ischemia/reperfusion injury, suggesting that ROS generation by MAO-B contributes to cardiac injury under stress conditions [79].

OXIDATIVE STRESS AND METABOLIC SWITCH IN CARDIAC DISEASES

Metabolomics has become a powerful research tool in cardiovascular disease as knowledge of the metabolic bases of CVDs advances. The ability to measure changes in metabolite concentrations and discover new biomarkers of different cardiovascular diseases has provided new insights into diagnoses and the development of new therapeutic approaches for CVDs. We highlight metabolic switches orchestrated by oxidative stress in cardiomyocytes cells, animal models, and human heart biopsies.

Oxidative Stress (OS) has been shown to play an essential role in the pathophysiology of the most severe cardiac diseases. As previously mentioned, a balance between the levels of Reactive Oxygen Species (ROS) and the antioxidant defenses is essential for cell health. Otherwise, excessive production of ROS that overcomes the antioxidant capacity causes damage to macromolecules, including protein, lipids, and DNA, and can trigger cell death [3,80-83]. However, although several signaling pathway studies and functional in vivo experiments have demonstrated the effects of ROS production in CVDs, scarce information is available about measurable changes in the metabolomic profile of cardiomyocytes undergoing oxidative stress.

Recently, in search of a model to explore the effects of oxidative stress, specifically in cardiomyocyte metabolism, Amaral et al. developed an *in vitro* platform to explore the metabolic switch induced by stressors agents on H9c2 cardiomyocytes through LC/MS untargeted metabolomics technique [84]. As a result, the authors depicted metabolic alterations of glucose, lipid, pyrimidine and purine biosynthesis, and glutathione pathways in cardiomyocytes exposed to H_2O_2. Such modulations will be discussed in the context of cardiac diseases.

Glucose metabolism

Glucose metabolism is essential for life maintenance. Carbohydrates, lipids, and proteins are ultimately broken down into glucose, the primary metabolic fuel of mammal cells and the precursor for synthesizing several cellular compounds. Glucose transport into cardiomyocytes is regulated by transmembrane Glucose Transporters (GLUTs) [85]. In the cytoplasm, glucose is converted to glucose-6-phosphate and oxidized to pyruvate, transported into mitochondria, where it will be oxidized by the Tricarboxylic Acid (TCA) cycle [86].

Several studies have reported that mitochondrial glucose oxidation is defective in the failing heart [87,88]. Ussher et al. demonstrated that in HF, pyruvate dehydrogenase activity is decreased, which reduces pyruvate oxidation by the TCA cycle. As a result, glycolysis is increased, elevating the circulating levels of lactate [89]. Likewise, Amaral et al. showed an upregulation of glycolysis metabolites such as Glucose-6-phosphate (2.01-fold), Glycerol-3-phosphate (0.8-fold), and Lactate (1.59-fold) upon H2O2 exposed cardiomyocytes, indicating a shift of energy metabolism to anaerobic glycolysis as an adaptive response to oxidative stress [84]. The authors also identified the accumulation of citrate levels (0.48-fold) and an upregulation of amino acid biosynthesis pathways, indicating dysfunction of the citric acid cycle and a shift toward amino acid biosynthesis [84,90].

Interestingly, Frezza et al. reported the action of HIF-1α factor during oxidative stress to promote the expression of genes involved in shifting the metabolism towards anaerobic glycolysis, impairing the citric acid cycle activity to couple to oxidative stress [91]. Furthermore, in a rat model, glucose oxidation rates were increased during compensated phases of cardiac hypertrophy, while during HF, glucose oxidation was downregulated [92,93]. Another interesting study was published by Ranjbarvaziri et al. [94]. In order to identify the functional components governing Hypertrophic Cardiomyopathy (HCM), the most common heritable cardiovascular disease [95,96], authors performed metabolomics and transcriptomics experiments on human heart samples. The metabolomics approach revealed alterations in carbohydrate metabolism, which significantly decreased in HCM. The levels of glucose, glycolysis intermediates (fructose 6-phosphate and phosphoenolpyruvic acid), pentose phosphate pathway metabolites (ribose 5-phosphate; ribulose 5-phosphate), and TCA cycle intermediates (malate, citrate, succinate) were downregulated, suggesting a global energetic decompensation.

Nowadays, it is accepted that alterations in glucose utilization vary depending on the etiology and severity of heart pathology, which possibly explains the discrepancy among the studies mentioned above [6].

Lipid metabolism

Several abnormalities in lipid metabolism have been identified in cardiovascular diseases. A free fatty acid crosses the sarcolemma and enters the cytoplasm, where it can be converted into Acyl-Coenzyme A (acyl-CoA). Thereafter, acyl-CoA is converted to acylcarnitine to enable its entry into mitochondria to be oxidized in the β-oxidization [86]. Changes in fatty acid oxidation rates or damage in mitochondrial β-oxidation can be reflected in acylcarnitines profiles since these metabolites are derivatives of fatty acyl-CoA [97].

In line with these findings, Amaral et al. recently demonstrated that L-carnitine was upregulated (1.9-fold) in H9c2 cardiomyocytes under oxidative stress [84]. Wang et al. suggested that L-carnitine protects cardiomyocytes against doxorubicin-induced oxidative stress and myocardial injury [98]. The upregulation of L-carnitine may be related to a reduction in intra-mitochondrial acetyl-CoA in response to mitochondria oxidative damage and reducing β-oxidation, as previously reported [99]. Hunter et al. demonstrated that the levels of acylcarnitines were increased among HF patients with preserved ejection fraction, and even higher levels were found in those with reduced ejection fraction [100].

Nevertheless, Bedi et al. found reduced levels of these compounds in end-stage HF patients compared to tissue from healthy ones [101]. Ranjbarvaziri et al. also described a decrease in the abundance of acylcarnitines in samples from HCM patients, suggesting a defect in converting free fatty acid to acylcarnitine. Consistently, mitochondrial carnitine O-acetyltransferase, which catalyzes the conversion of acyl-CoA to acylcarnitine, was reduced. Moreover, the expression of genes involved in fatty acid β-oxidation was reduced in relation to normal hearts [94]. Decreased myocardial acylcarnitines might indicate impaired mitochondrial function and reduced β-oxidation [102-104], which is in line with previous findings showing a reduction of fatty acid oxidation during more severe stages of HF [102,105]. This controversial finding among this set of studies may be explained by the inclusion or exclusion of diabetic patients, in which circulating acylcarnitines are often elevated, or by the severity of the cardiac diseases [6,88]. Future metabolomics studies considering aspects of subgroups or *in vitro* assays with isolated cardiomyocytes will contribute to clarifying these discrepancies.

Regarding the metabolites involved in the sphingolipid metabolism, Amaral et al. demonstrated an upregulation of sphingosine 1-phosphate (2.65-fold) and phytosphingosine (2.31-fold) in H9c2 cardiomyocytes under oxidative stress [84]. In line with these findings, Ranjbarvaziri et al. showed an upregulation of ceramide and sphingomyelin in HCM patients [94]. Ceramides, derived from sphingomyelin metabolism, seem to be involved in the pathogenesis of cardiac diseases, causing lipotoxicity, inflammation, and cell death. Genetic ablation or pharmacological inhibition of ceramide biosynthesis enzymes ameliorates cardiac diseases, such as hypertension and cardiomyopathy [49]. Metabolic profiling studies have further highlighted the potential of such lipids to risk stratification and as biomarkers of heart disease [106].

Pyrimidine and purine biosynthesis

Oxidative phosphorylation, oxygen consumption, and ATP production are reduced during heart failure [107]. Ranjbarvaziri et al. showed evidence of heart decompensation of energy metabolism with a decrease in ATP, ADP, and phosphocreatine in HCM patients [93]. The authors also described a reduction in oxidative phosphorylation capacity through ATP synthase and a lower expression of several other mitochondrial complexes. An upregulation of the Uncoupling Protein 2 (UCP2) was also described. Next, the authors demonstrated that AMPK, the main sensor of cellular energy status [108, 109], was activated in hypertrophic hearts. These data suggest that despite increased metabolic demand and activation of AMPK in HCM hearts, decreased energy supply contributes to energetic deprivation.

Interestingly, Amaral et al. described a downregulation of the products UDP, CDP, and AMP of the pyrimidine and purine metabolism in cardiomyocytes under oxidative stress [83]. The downregulation of AMP levels was shown to maintain AMPK in its inhibited form [109]. When activated by energetic stress, the AMPK inhibits ATP-consuming pathways and triggers ATP-producing pathways, such as fatty acid oxidation, glucose uptake, and glycolysis [110]. Amaral et al. suggest that oxidative stress induces a metabolic adaptation mechanism that allows cell survival and the reactivation of the anabolic pathways in H9c2 cardiomyocytes after oxidative stress. Such differences described in the literature may be related to the multifactorial components of cardiac diseases. Future metabolomic experiments using isolated cardiomyocytes urge to clarify the impact of pyrimidine and purine metabolism on the evolution of this class of diseases.

Glutathione metabolism

As mentioned above, glutathione metabolism is the first line of defense against ROS, playing a pivotal role in the cardiovascular system [111]. Several studies have associated CVDs with redox imbalances in the heart, such as hypertension and atherosclerosis, linked to polymorphisms of the enzymes of this pathway [37]. Ranjbarvaziri et al. showed that patients with Hypertrophic Cardiomyopathy present high rates of oxidative stress in hearts, characterized by a high cystine level, low glutathione (GSH) disponibility, and an increased GSSG/GSH ratio (oxidized glutathione/reduced glutathione) [93]. Amaral et al. also reported a significant reduction of glutathione metabolite in cardiomyocytes under oxidative stress [83]. Studies that target oxidative stress as a therapeutic target have shown that the best strategy to improve its efficacy is to enhance the endogenous antioxidant capacity, increasing GSH levels [82,112]. Despite the knowledge concerning the antioxidant action of glutathione metabolism, the functioning of this pathway and its impacts on CVDs needs to be further clarified.

DISCUSSION

Oxidative stress has been shown to play key roles in the pathophysiology of cardiovascular disease. Excessive ROS generation damages several biomolecules, impairing cellular metabolism [2-5]. Dysregulations of metabolic pathways due to excess ROS generation have been explored to characterize the metabolic fingerprint of cardiovascular disease. Emerging metabolomics technology allows the measurement of hundreds of metabolites in biological fluids, biopsies or cell cultures and thus provides significant information on potential new biomarkers for different cardiovascular conditions [113].

Several potential biomarkers have been discovered from current untargeted and targeted metabolomics approaches. Biomarkers can be defined as measurable biochemical species indicative of a biological process, pathogen or pharmacological response. In CVD, for example, high blood pressure is considered a biomarker for hypertension and troponins T and I for myocardial infarction [114]. However, for a molecule to be considered a biomarker, it must meet some plausibility criteria. In addition to the reproducibility of the experiments, *in vitro, in vivo* and/or *in silico* models should be used to establish a new biomarker [115]. However, the biggest challenges in this field lies, not only in metabolomics, but in all the techniques used in the search for biomarkers, in the variability of biological sample preparation and analysis methods, and in the multifactorial components of heart disease that vary in the population.

Although several in vivo experiments have demonstrated the effects of oxidative stress on CVDs, poor information is available on measurable changes in the metabolic profile of cardiomyocytes under oxidative stress. In this field, when metabolomics approaches are coupled to biochemical assays in a search for molecular alteration description, damage to mitochondrial DNA and modification of oxidizing enzymes are commonly described in cardiac diseases [55,56,60,61]. More recently, with the improvement of techniques, impacts of oxidative stress on overall heart metabolism have been explored [5]. For instance, studies have shown the impact of oxidative stress on the energy-yielding metabolism [116]. In order to maintain the needs of contractile elements and ion pumps, cardiomyocytes generate impressive amounts of ATP [6]. Besides oxidative phosphorylation, the creatine kinase system provides a faster mechanism to maintain ATP levels by rapidly transferring high-energy phosphates from phosphocreatine to ADP [5,117,118]. Evidence of energy metabolism dysregulation with a decrease in ATP, ADP, and phosphocreatine were shown in HCM patients [94]. Amaral et al. also showed a disruption of energy metabolism, with downregulation of the products UDP, CDP, and AMP of the pyrimidine and purine metabolism in H9c2 cells submitted to oxidative stress [84]. Several studies have shown that disruption of cardiac energy has a major role in heart failure [59,116,119].

Lactate also plays an important role in energy metabolism. Some studies have shown an increase in lactate levels in cardiac tissue under oxidative stress [84,89] due to the activation of anaerobic glycolysis. This event brings to light new functions of lactate that modulate lactate-sensitive genes involved in the regulation of cardiac muscle metabolism. Gabriel-Costa et al. [120,121] show evidence that elevated levels of lactate increase the expression of genes related to the lactate oxidation complex and NOX activity, resulting in ROS generation in cardiac muscle. These results suggest that metabolites derived from energy metabolism are high-potential candidates to cardiomyopathies biomarkers.

Carnitine is another metabolite quoted to be a new biomarker of oxidative stress in cardiomyocytes. Responsible for the transport of fatty acids into the mitochondria for beta oxidation, the increase of its levels may be related to disorders of fatty acid oxidation, resulting in energy loss production in the heart [84]. On the other hand, reduced levels of carnitine may be related to its consumption as a cardiac antioxidant [99,122]. Changes in its levels have been discussed to be a therapeutic biomarker, but it is still necessary to clarify the metabolic regulation of carnitine under stress conditions.

As for glutathione and its derivatives, the reduction in their levels during oxidative stress is remarkable, since they are key metabolites in antioxidant defense. It is likely that its function was exerted to reduce the effect of ROS on cardiac cells. However, as observed by Amaral et al. [84], the action of antioxidant species is not sufficient to prevent DNA damage and other changes in cardiac cells during oxidative stress. Despite this, Homma et al. [123] presents glutathione as a new candidate for therapeutic applications. Thus, to improve the therapeutic for cardiovascular diseases, more accurate diagnoses are required. To achieve this, metabolomics techniques can be used for the discovery of new biomarkers in heart disease and also to improve the understanding of the pathophysiology of diseases that affect the heart.

CONCLUSION

This review highlights recent advances in metabolomic profile applied to the characterization of the pathophysiology of the most relevant cardiac diseases, focusing on oxidative stress as a damaging agent. The advances in metabolomic platforms, predominantly based on NMR and mass spectrometry, enabled metabolomics to address the molecular mechanisms of cardiac diseases with implications for therapeutic efficacy and the discovery of new biomarkers for ameliorating the diagnostics. Furthermore, such techniques have enabled the description of shifts in the central metabolic pathways of cardiac cells under diverse oxidative stress conditions. We emphasized the discussion of metabolic switches in glucose, lipids, purine and pyrimidines, and glutathione metabolism, orchestrated by oxidative stress in cardiac diseases, and made a parallel with the results reached by H_2O_2-induced oxidative stress in H9c2 cardiomyocytes. The advances in the knowledge on cardiomyocyte metabolic regulation by oxidative stress will help to identify new biomarkers and opportunities to develop more effective ROS-based therapies.

ACKNOWLEDGMENTS

Authors would like to thank the Agency for the Improvement of Higher Education Personnel (CAPES) [Finance Code 001], National Council for Scientific and Technological Development

(CNPq) [grant number 42.3228/2018-8], and São Paulo Research Foundation (FAPESP) [grant number 2018/07383-6]. Original work from the author's laboratories were funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2014/50938-8) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (465699/2014-6).

AUTHOR CONTRIBUTIONS

AGA, HFC, and AMS wrote the manuscript. All authors revised and approved the submitted version.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Hermes-Lima M, Pereira B, Bechara EJ. Are free radicals involved in lead poisoning? Xenobiotica. 1991;21(8):1085-1090.
2. Touyz RM. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: What is the clinical significance? Hypertension. 2004;44(3):248-252.
3. Rodrigo R, Libuy M, Feliú F, Hasson D. Molecular basis of cardioprotective effect of antioxidant vitamins in myocardial infarction. BioMed Research International. 2013.
4. Deidda M, Noto A, Bassareo PP, Cadeddu Dessalvi C, Mercuro G. Metabolomic approach to redox and nitrosative reactions in cardiovascular diseases. Front Physiol. 2018;9:672.
5. Senoner T, Dichtl W. Oxidative stress in cardiovascular diseases: Still a therapeutic target? Nutrients. 2019;11(9):2090.
6. Müller J, Bertsch T, Volke J, Schmid A, Klingbeil R, Metodiev Y, et al. Narrative review of metabolomics in cardiovascular disease. J Thorac Dis. 2021;13(4):2532.
7. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol. 2014;24(10):453-462.
8. Uddin F, Rudin CM, Sen T. CRISPR gene therapy: Applications, limitations, and implications for the future. Front Oncol. 2020;10:1387.
9. Westermann L, Neubauer B, Köttgen M. Nobel Prize 2020 in Chemistry honors CRISPR: A tool for rewriting the code of life. Pflugers Arch. 2021;473(1):1-2.
10. Westermann L, Neubauer B, Köttgen M. Nobel Prize 2020 in Chemistry honors CRISPR: A tool for rewriting the code of life. Methods. 2021;473(1):1-2.
11. Fukuda IM, Pinto CF, Moreira CD, Saviano AM, Lourenço FR. Design of Experiments (DoE) applied to pharmaceutical and analytical Quality by Design (QD). Braz J Pharm Sci. 2018;54.
12. Diab S, McQuade DT, Gupton BF, Gerogiorgis DI. Process design and optimization for the continuous manufacturing of nevirapine, an active pharmaceutical ingredient for HIV treatment. Org. Process Res Dev. 2019;23(3):320-333.
13. Hartford A, Thomann M, Chen X, Miller E, Bedding A, Jorgens S, et al. Adaptive designs: Results of 2016 survey on perception and use. Ther Innov Regul Sci. 2020;54(1):42-54.
14. Karczewski KJ, Snyder MP. Integrative omics for health and disease. Nat Rev Genet. 2018;19(5):299-310.
15. Misra BB, Langefeld C, Olivier M, Cox LA. Integrated omics: Tools, advances and future approaches. J Mol Endocrinol. 2019;62(1):21-45.
16. Subramanian I, Verma S, Kumar S, Jere A, Anamika K. Multi-omics data integration, interpretation, and its application. Bioinform Biol Insights. 2020;14:1177932219899051.
17. Canuto GA, Costa JL, Da Cruz PL, Souza AR, Faccio AT, Klassen A, et al. Metabolômica: Definições, estado-da-arte e aplicações representativas. Quim Nova. 2018;41:75-91.
18. Fiehn O. Metabolomics the link between genotypes and phenotypes. Plant Mol Biol. 2002:155-171.
19. Fiehn O. Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks. Comp Funct Genomics. 2001;2(3):155-168.
20. Ren JL, Zhang AH, Kong L, Wang XJ. Advances in mass spectrometry-based metabolomics for investigation of metabolites. RSC advances. 2018;8(40):22335-22350.
21. Jonsson P, Gullberg J, Nordström A, Kusano M, Kowalczyk M, Sjöström M, et al. A strategy for identifying differences in large series of metabolomic samples analyzed by GC/MS. Anal Chem. 2004;76(6):1738-1745.
22. Cubbon S, Antonio C, Wilson J, Thomas-Oates J. Metabolomic applications of hilic–lc–ms. Mass Spectrom Rev. 2010;29(5): 671-684.
23. Barbosa CG, Gonçalves NS, Bechara EJ, Assunção NA. Potential diagnostic assay for cystinuria by capillary electrophoresis coupled to mass spectrometry. J Braz Chem Soc. 2013;24(4):534-540.
24. Feider CL, Krieger A, DeHoog RJ, Eberlin LS. Ambient ionization mass spectrometry: Recent developments and applications. Anal Chem. 2019;91(7):4266-4290.
25. Theiner S, Schoeberl A, Schweikert A, Keppler BK, Koellensperger G. Mass spectrometry techniques for imaging and detection of metallodrugs. Curr Opin Chem Biol. 2021;61:123-134.
26. Coskun O. Separation techniques: Chromatography. North Clin Istanb. 2016;3(2):156.
27. Sutherland K. Gas chromatography/mass spectrometry techniques for the characterisation of organic materials in works of art. Phys Sci Rev. 2019;4(6).
28. Maldaner L, Collins CH, Jardim IC. Fases estacionárias modernas para cromatografia líquida de alta eficiência em fase reversa. Quim Nova. 2010;33(7):1559-1568.
29. Gama MR, Da Costa Silva RG, Collins CH, Bottoli CB. Hydrophilic interaction chromatography. Trends Anal Chem. 2012;37:48-60.
30. Assunção NA, Bechara EJ, Simionato AV, Tavares MF, Carrilho E. Eletroforese capilar acoplada à espectrometria de massas (CE-MS): Vinte anos de desenvolvimento. Quim Nova. 2008;31(8):2124-2133.
31. Viberg P, Nilsson S, Skog K. Nanospray mass spectrometry with indirect conductive graphite coating. Anal Chem. 2004;76(14): 4241-4244.
32. Ma A, McDermaid A, Xu J, Chang Y, Ma Q. Integrative methods and practical challenges for single-cell multi-omics. Trends Biotechnol. 2020;38(9):1007-1022.
33. Bottje WG. Oxidative metabolism and efficiency: The delicate balancing act of mitochondria. Poult Sci. 2019;98(10):4223-4230.
34. Kowaltowski AJ, De Souza-Pinto NC, Castilho RF, Vercesi AE. Mitochondria and reactive oxygen species. Free Radic Biol Med. 2009;47(4):333-343.
35. Panth N, Paudel KR, Parajuli K. Reactive oxygen species: A key hallmark of cardiovascular disease. Adv Med Sci. 2016;2016.
36. Kayama Y, Raaz U, Jagger A, Adam M, Schellinger IN, Sakamoto M, et al. Diabetic cardiovascular disease induced by oxidative stress. Int J Mol Sci. 2015;16(10):25234-25263.
37. Matuz-Mares D, Riveros-Rosas H, Vilchis-Landeros MM, Vázquez-Meza H. Glutathione Participation in the Prevention of Cardiovascular Diseases. Antioxidants. 2021;10(8):1220.

38. Ezzati M, Obermeyer Z, Tzoulaki I, Mayosi BM, Elliott P, Leon DA. Contributions of risk factors and medical care to cardiovascular mortality trends. Nat Rev Cardiol. 2015;12(9):508-530.
39. Tzoulaki I, Elliott P, Kontis V, Ezzati M. Worldwide exposures to cardiovascular risk factors and associated health effects: Current knowledge and data gaps. Circulation. 2016;133(23):2314-2333.
40. Hermida N, Michel L, Esfahani H, Dubois-Deruy E, Hammond J, Bouzin C, et al. Cardiac myocyte β3-adrenergic receptors prevent myocardial fibrosis by modulating oxidant stress-dependent paracrine signaling. Eur Heart J. 2018;39(10):888-898.
41. Dubois-Deruy E, Cuvelliez M, Fiedler J, Charrier H, Mulder P, Hebbar E, et al. MicroRNAs regulating superoxide dismutase 2 are new circulating biomarkers of heart failure. Sci Rep. 2017;7(1):1-10.
42. Li JM, Gall NP, Grieve DJ, Chen M, Shah AM. Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. Hypertension. 2002;40(4):477-484.
43. Dai DF, Johnson SC, Villarin JJ, Chin MT, Nieves-Cintrón M, Chen T, et al. Mitochondrial oxidative stress mediates angiotensin II–induced cardiac hypertrophy and Gαq overexpression–induced heart failure. Circ Res. 2011;108(7):837-846.
44. Dai DF, Hsieh EJ, Liu Y, Chen T, Beyer RP, Chin MT, et al. Mitochondrial proteome remodelling in pressure overload-induced heart failure: The role of mitochondrial oxidative stress. Cardiovasc Res. 2012;93(1):79-88.
45. Ide T, Tsutsui H, Hayashidani S, Kang D, Suematsu N, Nakamura KI, et al. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. Circ Res. 2001 Mar 16;88(5):529-535.
46. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev. 2014;94(3):909-950
47. Poetsch AR. The genomics of oxidative DNA damage, repair, and resulting mutagenesis. Comput Struct Biotechnol J. 2020;18:207-219.
48. Tubbs A, Nussenzweig A. Endogenous DNA damage as a source of genomic instability in cancer. Cell. 2017;168(4):644-656.
49. Iliou A, Mikros E, Karaman I, Elliott F, Griffin JL, Tzoulaki I, et al. Metabolic phenotyping and cardiovascular disease: An overview of evidence from epidemiological settings. Heart. 2021 ;107(14): 1123-1129.
50. Lancel S, Qin F, Lennon SL, Zhang J, Tong X, Mazzini MJ, et al. Oxidative posttranslational modifications mediate decreased SERCA activity and myocyte dysfunction in Gαq-overexpressing mice. Circ Rec. 2010;107(2):228-232.
51. Steinberg SF. Oxidative stress and sarcomeric proteins. Circ. Res. 2013;112(2): 393-405.
52. Panday A, Sahoo MK, Osorio D, Batra S. NADPH oxidases: An overview from structure to innate immunity-associated pathologies. Cell Mol. Immunol. 2015;12(1):5-23.
53. Moris D, Spartalis M, Tzatzaki E, Spartalis E, Karachaliou GS, Triantafyllis AS,et al. The role of reactive oxygen species in myocardial redox signaling and regulation. Ann. Transl. Med. 2017;5(16):324.
54. Sag CM, Santos CX, Shah AM. Redox regulation of cardiac hypertrophy. J. Mol. Cell Cardiol. 2014;73:103-111.
55. Santos CX, Nabeebaccus AA, Shah AM, Camargo LL, Filho SV, Lopes LR. Endoplasmic reticulum stress and Nox-mediated reactive oxygen species signaling in the peripheral vasculature: Potential role in hypertension. Antioxid. Redox Signal. 2014;20(1):121-34.
56. Münzel T, Camici GG, Maack C, Bonetti NR, Fuster V, Kovacic JC. Impact of oxidative stress on the heart and vasculature: part 2 of a 3-part series. J Am. Coll Cardiol.;70(2):212-29
57. Gang C, Qiang C, Xiangli C, Shifen P, Chong S, Lihong L. Puerarin suppresses angiotensin II-induced cardiac hypertrophy by inhibiting NADPH oxidase activation and oxidative stress-triggered AP-1 signaling pathways. Pharm. Pharm. Sci. 2015;18(2):235-248.
58. Zhang Q, Tan Y, Zhang N, Yao F. Polydatin prevents angiotensin II-induced cardiac hypertrophy and myocardial superoxide generation. Exp. Biol. Med. 2015;240(10):1352-1361
59. Sciarretta S, Zhai P, Shao D, Zablocki D, Nagarajan N, Terada LS, et al. Activation of NADPH oxidase 4 in the endoplasmic reticulum promotes cardiomyocyte autophagy and survival during energy stress through the protein kinase RNA-activated-like endoplasmic reticulum kinase/eukaryotic initiation factor 2α/activating transcription factor 4 pathway. Circ. Res. 2013;113(11):1253-1264.
60. Nishino T, Okamoto K, Eger BT, Pai EF, Nishino T. Mammalian xanthine oxidoreductase–mechanism of transition from xanthine dehydrogenase to xanthine oxidase. FEBS J. 2008;275(13): 3278-3289.
61. Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, et al. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation. 2001;104(20):2407-2411.
62. Lee BE, Toledo AH, Anaya-Prado R, Roach RR, Toledo-Pereyra LH. Allopurinol, xanthine oxidase, and cardiac ischemia. J. Investig. Med. 2009;57(8):902-909.
63. Minhas KM, Saraiva RM, Schuleri KH, Lehrke S, Zheng M, Saliaris AP, et al. Xanthine oxidoreductase inhibition causes reverse remodeling in rats with dilated cardiomyopathy. Circ Res. 2006;98(2):271-279.
64. Peoples JN, Saraf A, Ghazal N, Pham TT, Kwong JQ. Mitochondrial dysfunction and oxidative stress in heart disease. Exp Mol Med. 2019;51(12):1-3.
65. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, et al. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. Cell. 2005;122(2):221-33.
66. Orsini F, Migliaccio E, Moroni M, Contursi C, Raker VA, Piccini D, et al. The life span determinant p66Shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates trans-membrane potential. J. Biol. Chem. 2004;279(24): 25689-25695.
67. D'Oria R, Schipani R, Leonardini A, Natalicchio A, Perrini S, Cignarelli A, et al. The role of oxidative stress in cardiac disease: from physiological response to injury factor. Oxid. Med. Cell Longev. 2020; 5732956.
68. Rota M, LeCapitaine N, Hosoda T, Boni A, De Angelis A, Padin-Iruegas ME, et al. Diabetes promotes cardiac stem cell aging and heart failure, which are prevented by deletion of the p66shc gene. Circ. Res. 2006;99(1):42-52.
69. Sverdlov AL, Elezaby A, Qin F, Behring JB, Luptak I, Calamaras TD, et al. Mitochondrial reactive oxygen species mediate cardiac structural, functional, and mitochondrial consequences of diet-induced metabolic heart disease. J Am Heart Assoc. 2016;5(1):e002555.
70. Jeong EM, Chung J, Liu H, Go Y, Gladstein S, Farzaneh-Far A, et al. Role of mitochondrial oxidative stress in glucose tolerance, insulin resistance, and cardiac diastolic dysfunction. J. Am Heart Assoc. 2016;5(5): e003046.
71. Niemann B, Chen Y, Teschner M, Li L, Silber RE, Rohrbach S. Obesity induces signs of premature cardiac aging in younger patients: The role of mitochondria. J Am Coll Cardiol. 2011;57(5):577-585.
72. Edmondson DE, Binda C, Wang J, Upadhyay AK, Mattevi A. Molecular and mechanistic properties of the membrane-bound

mitochondrial monoamine oxidases. Biochemistry. 2009;48(20): 4220-4230.

73. Maggiorani D, Manzella N, Edmondson DE, Mattevi A, Parini A, Binda C, et al. Monoamine oxidases, oxidative stress, and altered mitochondrial dynamics in cardiac ageing. Oxid Med Cell Longev. 2017.
74. Machado-Vieira R, Mallinger AG. Abnormal function of monoamine oxidase-A in comorbid major depressive disorder and cardiovascular disease: Pathophysiological and therapeutic implications. Mol Med Rep. 2012;6(5):915-922.
75. Manzella N, Santin Y, Maggiorani D, Martini H, Douin-Echinard V, Passos JF, et al. Monoamine oxidase-A is a novel driver of stress-induced premature senescence through inhibition of parkin-mediated mitophagy. Aging cell. 2018;17(5):e12811.
76. Santin Y, Sicard P, Vigneron F, Guilbeau-Frugier C, Dutaur M, Lairez O, et al. Oxidative Stress by Monoamine Oxidase-A Impairs Transcription Factor EB Activation and Autophagosome Clearance, Leading to Cardiomyocyte Necrosis and Heart Failure. Antioxid. Redox Signal. 2016;25(1):10-27.
77. Mialet-Perez J, Parini A. Cardiac monoamine oxidases: At the heart of mitochondrial dysfunction. Cell Death Dis. 2020; 11(1):1-3.
78. Kalaudercic N, Carpi A, Nagayama T, Sivakumaran V, Zhu G, Lai EW, et al. Monoamine oxidase B prompts mitochondrial and cardiac dysfunction in pressure overloaded hearts. Antioxid Redox Signal. 2014;20(2):267-280.
79. Heger J, Hirschhäuser C, Bornbaum J, Sydykov A, Dempfle A, Schneider A, et al. Cardiomyocytes-specific deletion of monoamine oxidase B reduced irreversible myocardial ischemia/reperfusion injury. Free Radic Biol Med. 2021;165:14-23.
80. Meneghini R. Iron Homeotasis, Oxidative Stress, and DNA Damage. Free Radic Biol Med. 1997; 23(5):783-792.
81. Alkadi H. A review on free radicals and antioxidants. Infect Disord. Drug Targets 2020;20(1):16-26.
82. Poljsak B, Šuput D, Milisav I. Achieving the balance between ROS and antioxidants: When to use the synthetic antioxidants. Oxid Med Cell Longev. 2013;956792.
83. Van Der Pol A, Van Gilst WH, Voors AA, Van Der Meer P. Treating oxidative stress in heart failure: Past, present and future. Eur J Heart Fail. 2019;21(4):425-435.
84. Amaral AG, Moretto IA, Zandonadi FS, Zamora-Obando HR, Rocha I. Sussulini A. Comprehending Cardiac Dysfunction by Oxidative Stress: Untargeted Metabolomics of in vitro Samples. Front Chem. 2022;10:836478.
85. Robinson R, Robinson LJ, James DE, Lawrence JC. Glucose transport in L6 myoblasts overexpressing GLUT1 and GLUT4. J Biol Chem. 1993;268(29):22119-22126.
86. Schwenk RW, Luiken JJFP, Bonen A, Glatz JFC. Regulation of sarcolemmal glucose and fatty acid transporters in cardiac disease. Cardiovasc. Res. 2008;79(2):249-258.
87. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. Physiol Rev. 2005;85(3): 1093-1129.
88. Gupte AA, Hamilton DJ, Cordero-Reyes AM, Youker KA, Yin Z, Estep JD. et al. Mechanical unloading promotes myocardial energy recovery in human heart failure. Circ Cardiovasc Genet. 2014;7(3): 266-276.
89. Ussher J R, Elmariah S, Gerszten RE, Dyck JR. The emerging role of metabolomics in the diagnosis and prognosis of cardiovascular disease. J Am Coll Cardiol. 2016;68(25):2850-2870.
90. Mullarky E, Cantley LC, Nakao K, Minato N, Uemoto S. Diverting glycolysis to combat oxidative stress in: Innovative medicine: Basic research and development. Springer;2015.
91. Frezza C, Zheng L, Tennant DA, Papkovsky DB, Hedley BA, Kalna G, et al. Metabolic profiling of hypoxic cells revealed a catabolic signature required for cell survival. PLoS One. 2011;6(9):e24411.
92. Degens H, Brouwer KFJ, Gilde AJ, Lindhout M, Willemsen HM, Janssen BJ, et al. Cardiac fatty acid metabolism is preserved in the compensated hypertrophic rat heart. Basic Res Cardiol. 2006; 101(1):17-26.
93. Doenst T, Pytel G, Schrepper A, Amorim P, Färber G, Shingu Y, et al. Decreased rates of substrate oxidation ex vivo predict the onset of heart failure and contractile dysfunction in rats with pressure overload. Cardiovasc Res. 2010; 86(3):461-470.
94. Ranjbarvaziri S, Kooiker KB, Ellenberger M, Fajardo G, Zhao M, Roest ASV, et al. Altered cardiac energetics and mitochondrial dysfunction in hypertrophic cardiomyopathy. Circulation. 2021;144(21): 1714-1731.
95. Maron BJ, Maron MS. Hypertrophic cardiomyopathy. Lancet. 2013;381(9862):242-255.
96. Semsarian C, Ingles J, Maron MS, Maron BJ. New perspectives on the prevalence of hypertrophic cardiomyopathy. J Am Coll Cardiol. 2015;65(12):1249-1254.
97. Bain JR, Stevens RD, Wenner BR, Ilkayeva O, Muoio DM, Newgard CB. Metabolomics applied to diabetes research: Moving from information to knowledge. Diabetes. 2009;58(11):2429-2443.
98. Wang ZY, Liu YY, Liu GH, Lu HB, Mao CY. L-Carnitine and heart disease. Life Sci. 2018; 194: 88-97.
99. Mate A, Miguel-Carrasco JL, Vázquez CM. The therapeutic prospects of using L-carnitine to manage hypertension-related organ damage. Drug Discov. 2010;15(11-12):484-492.
100. Hunter WG., Kelly JP, McGarrahill RW, Khouri, MG, Craig D, Haynes C, et al. Metabolomic Profiling Identifies Novel Circulating Biomarkers of Mitochondrial Dysfunction Differentially Elevated in Heart Failure With Preserved Versus Reduced Ejection Fraction: Evidence for Shared Metabolic Impairments in Clinical Heart Failure. J Am Heart Assoc. 2016;5(8), e003190.
101. Bedi Jr KC, Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, et al. Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. Circulation. 2016;133(8):706-716.
102. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. Physiol Rev. 2010;90(1):207-258.
103. Rosenblatt-Velin N, Montessuit C, Papageorgiou I, Terrand J, Lerch R. Postinfarction heart failure in rats is associated with upregulation of GLUT-1 and downregulation of genes of fatty acid metabolism. Cardiovasc Res. 2001;52(3):407-416.
104. Sack MN, Rader TA, Park S, Bastin J, McCune SA, Kelly DP. Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. Circulation. 1996;94(11):2837-2842.
105. Taegtmeyer H, Young ME, Lopaschuk GD, Abel ED, Brunengraber H, Darley-Usmar V, et al. Assessing cardiac metabolism: A scientific statement from the American Heart Association. Circ Res. 2016;118(10):1659-1701.
106. Chen H, Wang Z, Qin M, Zhang B, Lin L, Ma Q, et al. Comprehensive Metabolomics Identified the Prominent Role of Glycerophospholipid Metabolism in Coronary Artery Disease Progression. Front Mol Biosci. 2021;8.
107. Neubauer S. The failing heart: An engine out of fuel. N Engl J Med. 2007 ;356(11):1140-1151.
108. Garcia D, Shaw RJ. AMPK: Mechanisms of cellular energy sensing and restoration of metabolic balance. Mol Cell. 2017;66(6):789-800.
109. Lin SC, Hardie DG, AMPK: Sensing glucose as well as cellular energy status. Cell Metab. 2018;27(2):299-313.

110. Lipovka Y, Konhilas JP. AMP-activated protein kinase signalling in cancer and cardiac hypertrophy. Cardiovasc Pharm. 2015;4(3).

111. Rochette L, Lorin J, Zeller M, Guilland JC, Lorgis L, Cottin Y, et al. Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: Possible therapeutic targets? Pharmacol Ther. 2013;140(3):239-257.

112. Van Der Pol A, Gil A, Silljé HH, Tromp J, Ovchinnikova ES, Vreeswijk-Baudoin I, et al. Accumulation of 5-oxoproline in myocardial dysfunction and the protective effects of OPLAH. Sci Transl Med. 2017;9(415):8574.

113. Levy D, Larson MG, Vasan RS, Kannel WB, Ho KK. The progression from hypertension to congestive heart failure. JAMA. 1996;275(20):1557-1562.

114. Borges LP, Jesus RCS, Moura RL. Utilização de biomarcadores cardíacos na detecção de infarto agudo do miocárdio. REAS. 2019;11:e940.

115. Zamora-Obando HR, Godoy AT, Amaral AG, Mesquita AS, Simões BE, Reis HO. Biomarcadores moleculares de doenças humanas: conceitos fundamentais, modelos de estudo e aplicações clínicas. Quim. 2022;1-16.

116. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4:180-183.

117. Wallimann T. Bioenergetics. Dissecting the role of creatine kinase. Curr Biol. 1994;4(1):42-46.

118. Ingwall JS, Atkinson DE, Clarke K, Fetters JK. Energetic correlates of cardiac failure: Changes in the creatine kinase system in the failing myocardium. Eur Heart J. 1990;11:108-115.

119. Taegtmeyer H. Cardiac metabolism as a target for the treatment of heart failure. Circulation. 2004; 110:894-896.

120. Gabriel-Costa D, Cunha TF, Behcara LRG, Fortunato RS, Bozi LHM, Coelho MA. Lactate up-regulates the expression of lactate oxidation complex-related genes in left ventricular cardiac tissue of rats. PLoS ONE. 2015;10(5):e0127843.

121. Gabriel-Costa D, Cunha TF, Paixão NA, Fortunato RS, Rego-Monteiro ICC, Barreto-Chaves ML M, Brum PC. Lactate-upregulation of lactate oxidation complex-related genes is blunted in left ventricle of myocardial infarcted rats. Braz J Med Biol. 2018;51(11):e7660.

122. Coelho CF, Mota JF, Bragrança E, Burini RC. Aplicações clínicas da suplementação de L-carnitina. Ver Nutr. 2005;18(5):651-659.

123. Homma T, Fujii J. Application of glutathione as anti-oxidative and anti-aging drugs. Curr Drug Metab. 2015;16(7):560-571.