Hydrogen sulfide (H_2S) attenuates lipotoxicity and cardiac cell senescence by regulating protein acetylation

by

Ruihuan Yu

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Thesis Examiners/Examinateurs de thèse:

Dr. Guangdong Yang
(Supervisor/Directeur(trice) de thèse)

Dr. Thomas Merritt
(Committee member/Membre du comité)

Dr. Amadeo Parissenti
(Committee member/Membre du comité)

Dr. Alain Simard
(Committee member/Membre du comité)

Dr. Karmin O
(External Examiner/Examinateur externe)

Approved for the Faculty of Graduate Studies
Approuvé pour la Faculté des études supérieures
Tammy Eger, PhD
Vice-President Research (Office of Graduate Studies)
Vice-rectrice à la recherche (Bureau des études supérieures)
Laurentian University / Université Laurentienne

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Abstract

Hydrogen sulfide (H_2S) is recently recognized as a novel gasotransmitter. H_2S can be endogenously generated from cysteine in mammalian tissues, and cystathionine gamma-lyase (CSE) is a critical enzyme generating H_2S in the cardiovascular system. Increasing evidence suggests that interference in H_2S production is related to heart diseases. Obesity is a leading risk factor for heart dysfunctions by interrupting lipid metabolism. In the current work, the regulatory roles of the CSE/H_2S system on lipid overload-induced lipotoxicity and cardiac senescence were explored. Here, it was found that incubation of H9C2 rat cardiomyocyte cells with a lipid mixture inhibited cell viability and promoted the cellular accumulation of lipids, formation of reactive oxygen species, mitochondrial dysfunctions, and lipid peroxidation; all of these could be reversed through incubation with the exogenously applied NaHS (the H_2S donor). Further data revealed that H_2S protected H9C2 cells from lipid overload-induced senescence by altering the expression of genes related to lipid metabolism and inhibiting both the production of acetyl-CoA and the level of protein acetylation. *In vivo*, knockout of the CSE gene strengthened cardiac lipid accumulation, protein acetylation, and cellular ageing in the mice fed a high-fat diet. Taken together, the CSE/H_2S system is essential for maintaining lipid homeostasis and cellular senescence in heart cells under lipid overload. The CSE/H_2S system would serve as a target for preventing and treating obesity and age-related heart diseases.

Keywords: H_2S, cystathionine gamma-lyase, lipid metabolism, lipotoxicity, senescence, protein acetylation

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List of abbreviation

3MST: 3-mercaptopyruvate sulfurtransferase

AceCS1: Cytoplasmic acetyl-CoA synthetase 1

ATP-CL: ATP-citrate lyase

ACOX1: Acyl-CoA oxidase 1

ACSL1: Long-chain acyl-CoA synthetase 1

ANF: Atrial natriuretic factor

ApoB: Apolipoprotein B

BNP: Brain natriuretic peptide

CBS: Cystathionine beta-synthase

CSE: Cystathionine gamma-lyase

KO: Knockout

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

PPARα: Peroxisome proliferator-activated receptor alpha

PPG: DL-propargylglycine

SAHA: Suberoylanilide hydroxamic acid

SREBP1: Sterol regulatory element-binding protein 1

VLDL: Very low-density lipoprotein

WT: Wild type

1. Introduction

1.1. H_2S signaling in cardioprotection

1.1.1. Endogenous production of H_2S

Hydrogen sulfide (H_2S) is a small molecule that can freely cross the cell membrane (Wang, 2012). H_2S is considered to be a novel gaseous mediator, similar to carbon monoxide and nitric oxide (Wang, 2012; Linden et al., 2010; Leslie, 2008). The endogenous H_2S can be constitutively generated in mammalian tissues through enzymatic and/or nonenzymatic pathways (kolluru et al., 2013). The 3-mercapto pyruvate sulfurtransferase (MST), cystathionine β synthase (CBS), and the cystathionine γ lyase (CSE) are three critical enzymes for determining the level of H_2S generation in mammalian tissues (Ahmad et al., 2016; Shibuya et al., 2009; Yang et al., 2018). CSE and CBS account for most of the endogenous H_2S generation using L-cysteine and homocysteine as substrates. The principal mechanism of H_2S formation from CBS is through the β-replacement reaction with cysteine and water to create serine (Chen et al., 2004; Kabil and Banerjee, 2014; Kabil et al., 2011; Sbodio et al., 2019). Through an α, β elimination reaction, L-cysteine can also be used by CSE to generate H_2S. Nevertheless, when high homocysteine levels exist, the γ-replacement between two homocysteine molecules is the essential reaction for the formation of H_2S (Kabil et al., 2011) (**Equation 1**). CSE and CBS are the enzymes dependent on pyridoxal-5-phosphate (PLP) and appear to be exclusively cytosolic (Allsop and Watts, 1975; Donnarumma et al., 2017). MST is another H_2S-generating enzyme, which interacts with the cysteine aminotransferase (CAT) to produce H_2S (Shibuya et al., 2009). Different from CSE

and CBS, CAT and MST exist in both the cytoplasm and mitochondria, and about two-thirds of the 3-MST is present in mitochondria (Lavu et al., 2011) (**Fig. 1**).

Equation 1*:*

L-cysteine + H_2O → H_2S + L-pyruvate + NH3

L-Homocysteine + L-Homocysteine → H_2S + L-Homolanthionine

Experimental evidence suggests the distributions of these enzymes are tissue-specific. CBS is regarded as the main enzyme producing H_2S in the brain and nervous system (Abe et al., 1996), and it has also been confirmed to be present in other tissues (Teng et al., 2013; Szabo et al., 2013). CSE is the major enzyme generating H_2S in the cardiovascular system and is also exists in the liver, kidney, and gastrointestinal tract (Fiorucci et al., 2006; Guo et al., 2012; Martin et al., 2010; Patel et al., 2009; Xin et al., 2016; Yang et al., 2005; Zhao et al., 2001). Different from CBS, CSE is not expressed constitutively in cells, and the expression of CSE is highly induced via a series of stimuli, such as oxidative stress, endoplasmic reticulum (ER) stress, hyperhomocysteinemia and a nutritional deficiency (Hassan et al., 2012; Hourihan et al., 2013; Sbodio et al., 2019). The CAT and MST jointly contribute to H_2S generation in the vascular endothelium and brain (Manna et al., 2014; Tomita et al., 2016). Nonenzymatic generation of H_2S occurs *via* the reaction of thiol or thiol-containing compounds with other molecules, such as the reduction of dietary inorganic polysulfides by

glutathione (GSH) or the hydrolysis of inorganic sulphide salts (namely, sodium hydrogen sulphide [NaHS] or sodium sulphide [Na_2S]) with water (Benavides et al., 2007; Powell et al., 2018).

Previous reports suggest that the concentration of H_2S in cells varies from high nanomolar to lower micromolar concentrations, but in most tissues, the H_2S steady-state concentration may be in a low nanomolar range (Furne et al., 2008; Sokolov et al., 2021). Under normal physiological conditions, H_2S can maintain its levels through a variety of catabolic pathways. The H_2S elimination processes contain oxidation, methylation, and clearance through expiration and excretion (Kimura, 2012, 2013; Shibuya et al., 2013). Nevertheless, in the above processes, the principal pathway is oxidation, and mitochondria are very effective in this process (Goubern et al., 2007).

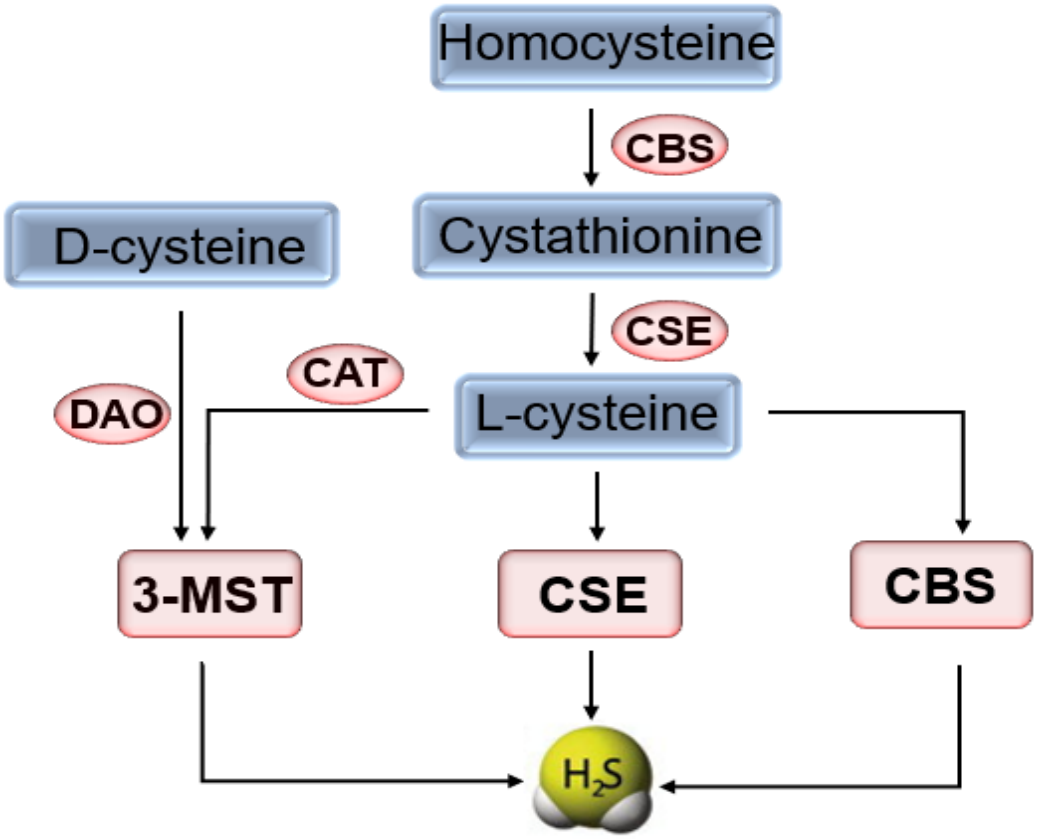

Fig. 1. Enzymatic contribution of endogenous H_2S in the mammalian tissues (Dugbartey, 2017).

1.1.2. Physiological roles of H_2S in mammalian tissue

H_2S has been confirmed to be present in a variety of organs and tissues of mammals as a new gasotransmitter and possesses an extensive range of physiological effects in mammalian tissues (Gadalla and Snyder, 2010; Luo et al., 2020). Various researches have demonstrated that H_2S participates in a variety of pathological and physiological processes, including antioxidant defence, autophagy, apoptosis, differentiation of stem cells, and cellular

senescence (Albertini et al., 2012; Baskar et al., 2008; Kimura, 2013; Sun et al., 2019; Wang et al., 2020; Wu et al., 2018; Yang et al., 2006; Yang and He, 2019) (**Fig. 2**). In addition, accumulating evidence suggests that H_2S modulates different cellular functions in a cell- and tissue-specific manner (Szabo and Papapetropoulos, 2017).

H_2S can interfere with central metals of iron-heme proteins, as a kind of antioxidant, to remove reactive nitrogen species (RNS) and reactive oxygen species (ROS). H_2S post-translationally modifies protein cysteine residues via S-persulfidration, further influencing the function of various proteins (Filipovic, 2015; Filipovic et al., 2018; Kimura, 2020; Powell et al., 2018; Sen, 2017).

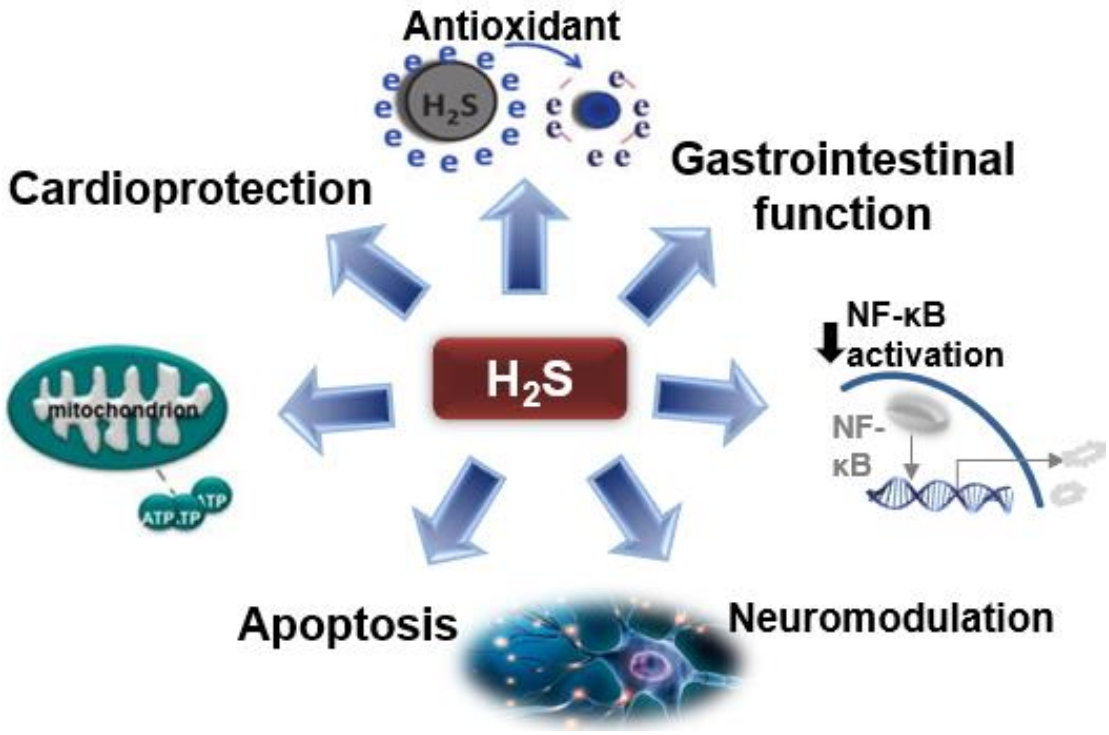

Fig. 2. Physiological roles of H_2S in mammalian tissue (Kabil et al., 2014).

1.1.3. The cardioprotective effects of H_2S

H_2S is principally generated through CSE using L-cysteine as a substrate in the vascular system and heart. Previously published experimental studies exhibited that H_2S is generated in the circulation at micromolar levels (between 10 and 100 μM) and helps maintain the physiological functions of the heart. It has been shown that H_2S can decrease blood pressure, cause vasodilation *in vitro*, relax vascular smooth muscle, and inhibit the interaction between endothelial cells and leukocytes in circulation (Pryor et al., 2006). Furthermore, H_2S is an effective antioxidant, which can augment tissues' antioxidant defence capacities in chronic diseases. Moreover, the treatment of H_2S can reverse cardiomyocyte apoptosis caused by a variety of stressors, including ischemia/reoxygenation (I/R), H_2O_2, high glucose (Wu et al., 2015; Xu et al., 2015).

In the past several years, growing evidence indicates that a dysregulation of endogenous H_2S production can promote a variety of heart diseases. The exogenous H_2S administration or the regulation of signalling pathways associated with endogenous H_2S has shown to produce a protective effect in various models of cardiac injury (Donnarumma et al., 2017). For example, decreased expression of CSE and lowered H_2S generation was shown in human samples of hypertrophic myocardium (Meng et al., 2016). The supplementation of H_2S was shown to prevent myocardial hypertrophy in spontaneously hypertensive rats by

changing the transcription factor KLF5 (Meng et al., 2016). Similarly, animal experiments revealed that the formation of endogenous H_2S in myocardial cells was remarkably reduced during ischemia (Donnarumma et al., 2017). Both *in vivo* and *in vitro* investigations have demonstrated a protective effect of H_2S therapy on the myocardial injury from ionizing radiation (Bibli et al., 2015; Hu et al., 2016). Further investigation revealed that H_2S-induced cardioprotection in cardiomyocytes is due to inflammation inhibition via blocking transactivation of NF-κB, stimulation of antioxidant pathways through targeting at Nrf2 transcription factor, and heart function protection via activating sarcolemmal K_{ATP} channels (Jin et al., 2017; Pan et al., 2006). A report by Kar *et al.* first elucidated that exercise could prevent cardiac dysfunction caused by a high-fat diet (HFD) in mice by inhibiting myocardial remodelling and protecting mitochondrial function, possibly via H_2S-mediated cardioprotection (Kar et al., 2019) (**Fig. 3**).

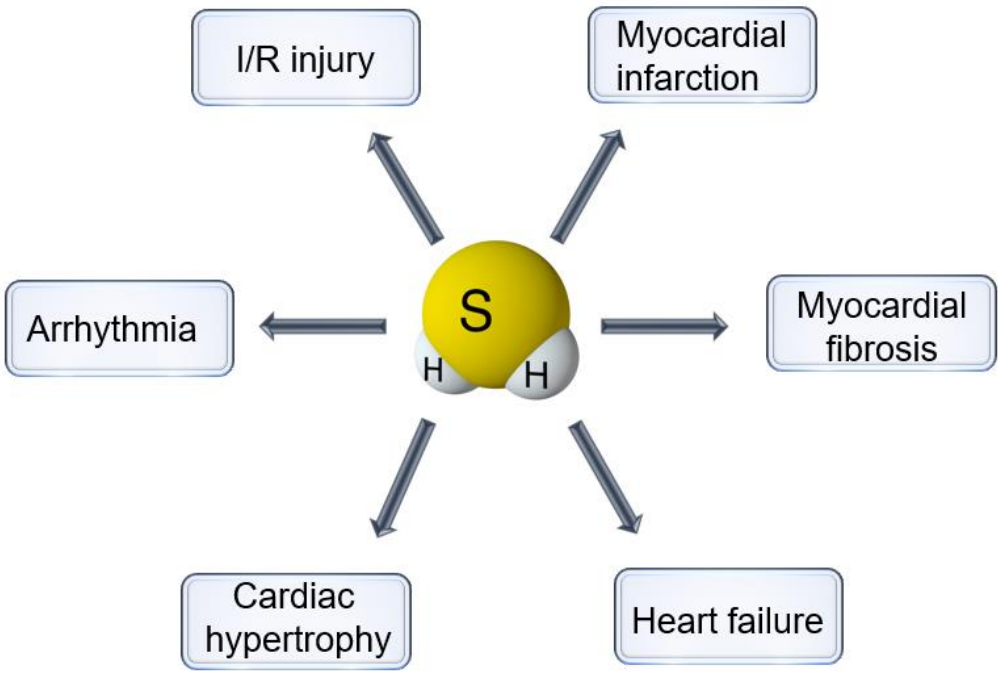

Fig. 3. The protective effects of H_2S on the heart (Shen et al., 2015).

1.2. Obesity, lipotoxicity and heart dysfunction

1.2.1. Obesity and heart dysfunction

Obesity, primarily due to nutrient intake exceeding nutrient expenditure, is a serious health concern in the past three decades (Sletten et al., 2018). The pathogenesis of obesity stems from many factors, including lifestyle, epigenetics, and genetics (Albuquerque et al., 2015; Hopkins and Blundell, 2016; MacLean et al., 2017; Parrillo et al., 2016; Raciti et al., 2017; Schwartz et al., 2017). Severe obesity is related to an increased risk of adverse health consequences that also influence medical costs, life quality, and life expectancy.

Severe obesity, defined as a body mass index equal to or greater than 40/kg/m^2, can lead to cardiac structure and function changes. Primary myocardial alterations on account of obesity include left atrial enlargement, left ventricular (LV) hypertrophy, subclinical impairment of LV systolic and diastolic function, cardiomyopathy, arrhythmias, and valvular heart diseases enhancing the risk of heart failure (Abel et al., 2008). These changes lead to high cardiovascular mortality in the world (Abel et al., 2008; Borlaug et al., 2010; Ebong et al., 2014; Eckel et al., 2002; Haykowsky et al., 2014; Kang, 2013; Kitzman and Shah, 2016;

Kopelman, 2000; Maeder et al., 2010; Obokata et al., 2017; Park and Goldberg, 2012; Paulus and Tschöpe, 2013; Shah et al., 2016; Upadhya et al., 2015) (**Fig. 4**).

Prior studies have confirmed that the changes in ventricular function and cardiac structure caused by obesity are due to hemodynamic changes (Alpert, 2001; Alpert et al., 2014; Wong and Marwick, 2007). Recent clinical trials and experimental studies utilizing animal models strongly suggest that obesity-related lipotoxicity may also have adverse effects on cardiac function and structure (Abel et al., 2008; Briones et al., 2012; Even et al., 2014; Haykowsky et al., 2014; Kang, 2013; Normandin et al., 2015; Obokata et al., 2017; Parisi et al., 2016). In simplistic terms, cardiac lipotoxicity is a process characterized by the excessive accumulation of lipid intermediates and lipids in the heart (Abdurrachim et al., 2014). This kind of accumulation may result in cellular dysfunction and even cell death, ultimately causing the dysfunction of the whole organ (Alpert et al., 2018; Schulze et al., 2016).

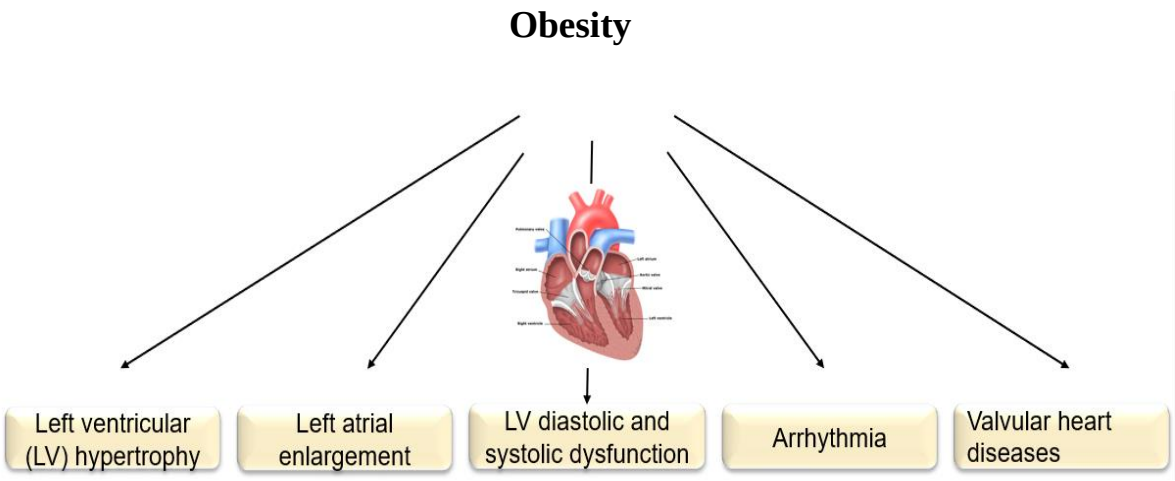

Fig. 4. Obesity and heart dysfunction (Castañeda et al., 2019).

1.2.2. Heart lipid metabolism

The metabolism of lipids is an intricate process composed of the uptake, synthesis, oxidation, and transport of lipids. For all the lipid types, fatty acids are the most abundant, so the lipid content in tissue and plasma is decided via the availability of free fatty acids (FFA) (Vega and Kelly, 2017).

The extracellular FFAs enter the cardiac myocyte via a variety of lipid transporters, such as the cluster of differentiation 36 (CD36), and then go through acyl-CoA oxidase (ACOX1) mediated β-oxidation in mitochondria, resulting in the release of acetyl-CoA (Vega and Kelly, 2017). Mitochondrially generated acetyl-CoA can be converted to citrate, which can then be exported from mitochondria to the cytosol, and converted to acetyl-CoA through ATP citrate lyase (ATP-CL) (Fushimi et al., 2006). Cytoplasmic acetyl-CoA can also originate from the metabolite acetate, which comes from a variety of intracellular and extracellular sources, including intestinal microbial metabolism, food digestion, acetylated protein deacetylation and alcohol oxidation (Mews et al., 2017). Acetate can be employed to produce the acetyl-CoA via acyl-CoA synthetase (AceCS1). Then acetyl-CoA formed in the cytosol can participate in the generation of de novo fatty acids through fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) (Todoric et al., 2020). Besides lipid catabolism, triglycerides (TAGs) are formed via esterifying fatty acids to glycerol; these TAGs are then

placed in lipid droplets (Lee et al., 2020). The stored energy can be mobilized to mitochondria by lipophagy or lipolysis, breaking down lipid droplets (D'Souza et al., 2016; Matey-Hernandez et al., 2018) (**Fig. 5**). Lipid metabolism significantly affects cellular energy demands and provides crucial components for biosynthetic pathways and redox homeostasis.

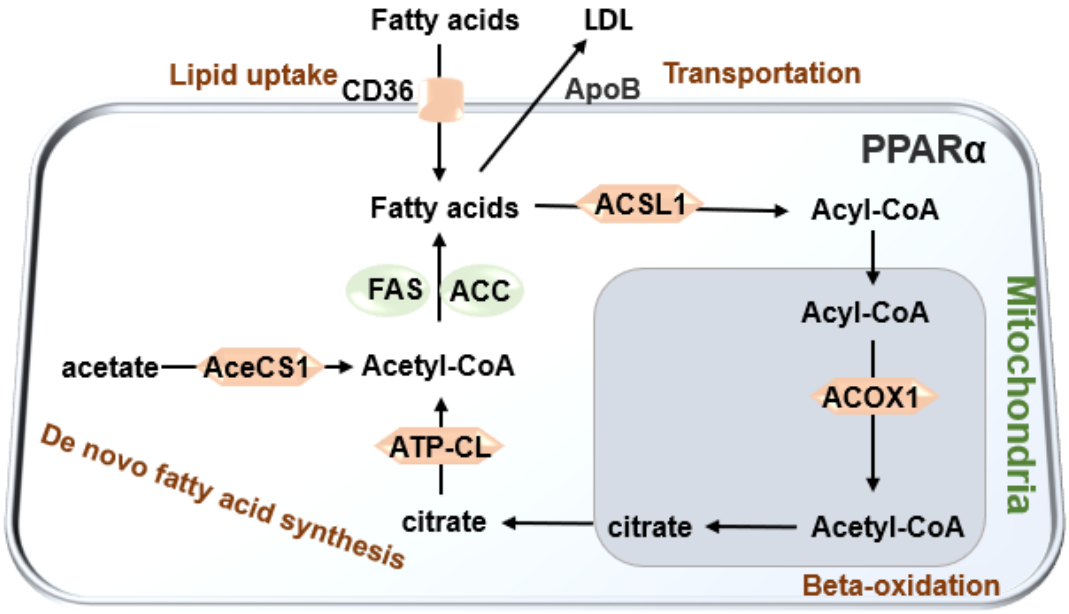

Fig. 5. Heart lipid metabolism (Yi et al., 2018).

1.2.3. Obesity and lipotoxicity

Obesity in patients and experimental animals is often accompanied by abnormal lipid metabolism, which causes lipid over deposition in fat tissues and eventually results in high circulating TAGs and fatty acids levels, causing an enhanced uptake of myocardial fatty acids and strong lipid oxidation (Buchanan et al., 2005; Lopaschuk et al., 1994; Luiken et al., 2001; Young et al., 2002). The excess fatty acids absorbed through the myocardium are mainly used

for the storage of TAGs or energy metabolism via β-oxidation of fatty acids in the mitochondria (Ji et al., 2017). The heart's ability to store lipids is limited, so excess lipids in the heart contribute to lipotoxicity (Chiu et al., 2001; Gaggini et al., 2015; Li et al., 2010). Lipotoxicity can adversely affect multiple cellular processes, including the increased generation of reactive oxygen species (ROS) and subsequent mitochondrion dysfunction, reduced ATP formation, and possibly reduced cell viability (Aurich et al., 2013; DeFronzo, 2010; van de Weijer et al., 2011). All of these contribute to heart disease pathogenesis related to obesity (**Fig. 6**).

More fatty acid delivery to cardiac cells and subsequent β-oxidation is conducive to the activation of serine/threonine kinases, which can inhibit the signalling of insulin through its receptor and the insulin receptor substrate (IRS), ultimately leading to decreased uptake of glucose. As a result, the obese heart finally loses metabolic flexibility and relies on fatty acid oxidation to produce ATP. Since the efficiency of fatty acids acting as energy substrates is about 10 to 12% lower than that of glucose in producing ATP at each consumption of oxygen, the high oxidation rate of fatty acids can decrease heart efficiency through uncoupling proteins (UCPs), which consume the mitochondrial proton gradient and cause mitochondrial uncoupling of ATP generation. It is worth noting that in *db*/*db* and *ob*/*ob* mice, the reduction of cardiac efficiency by changing the primary substrate for ATP generation precedes systolic cardiac dysfunction (Buchanan et al., 2005). Moreover, the increased oxidation rate of fatty acids and the consequent increase in the transfer of reducing equivalents to the electron

transport chain favour ROS generation in the heart. Animal researchers have strongly confirmed that when the FA supply exceeds the FA oxidation capacity of mitochondria, the result is obesity---leading to intracellular accumulation of lipids and lipid intermediates (Goldberg et al., 2012; Riehle and Abel, 2016). The outcomes of lipotoxicity are cardiomyocyte senescence and apoptosis, myocardial fibrosis, mitochondrial dysfunction, eventually leading to heart dysfunction and failure (Harayama and Riezman, 2018; Riehle and Abel, 2016).

Several animal experiments indicate a statistically significant close correlation between cardiac dysfunction and the accumulation of lipids in heart tissue (Kitzman and Shah, 2016; Wende and Abel, 2010). Marked myocardial triglyceride accumulation was observed in genetically obese rats, which could be related to the dysfunction of LV and cardiac hypertrophy (Drosatos and Schulze, 2013; McGavock et al., 2006; Wende and Abel, 2010; Wende et al., 2012). Furthermore, high-fat diet (HFD)-fed mice had elevated myocardial lipid accumulation and decreased LV strain (Hankiewicz et al., 2010). Previous studies also found that lipid overload in obesity and diabetes mellitus could cause heart dysfunction by facilitating acetylation of the fission protein Drp1 at K642, thus stimulating mitochondrial dysfunction and ROS generation (Hu et al., 2020). The above findings suggest that reducing fatty acid accumulation in the heart may be a strategy for preventing lipotoxic heart dysfunction.

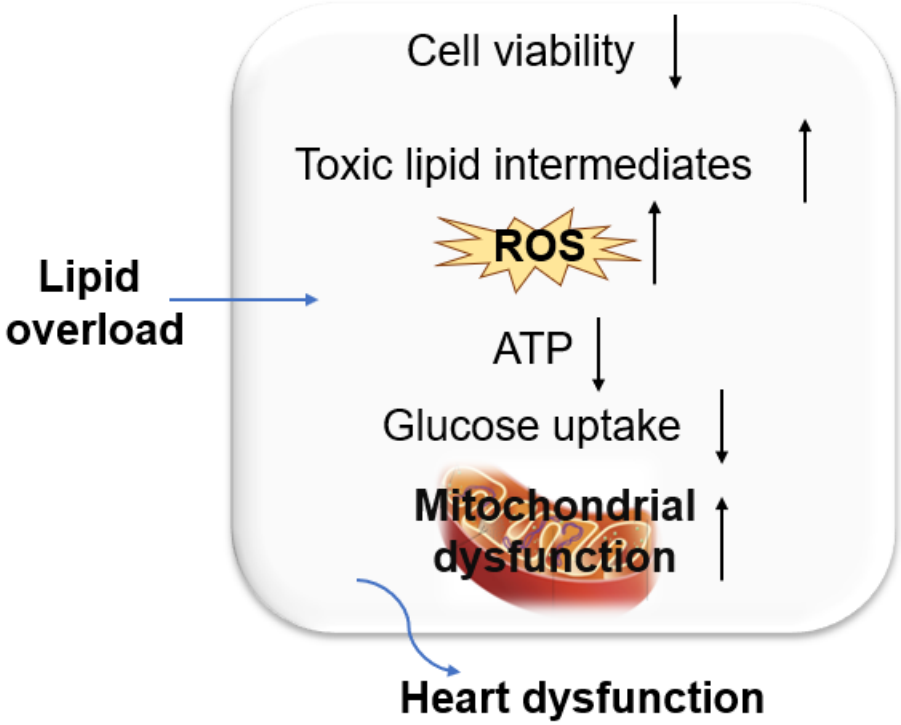

Fig. 6. Obesity and lipotoxicity in the heart (Koliaki et al., 2019).

1.3. Mitochondrial dysfunction, oxidative stress, lipid peroxidation, acetylation, and senescence

1.3.1. Oxidative stress

Oxidative stress occurs when there is an imbalance between oxidant and antioxidant defence capacities, resulting in the excessive presence of ROS in the cells, such as hydroxyl superoxide anion (O^{2-}), hydroxyl radical (OH^{-}), hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^{-}$) (Cheng et al., 2017; Vakifahmetoglu-Norberg et al., 2017) (**Fig. 7**). Under normally functioning physiological conditions, endogenous enzymatic and non-enzymatic antioxidant

systems can effectively reduce the level of ROS produced during cell oxidation to protect the cells from the harmful influences of high ROS concentrations (Moldogazieva et al., 2019; Niki, 2016; Valko et al., 2007).

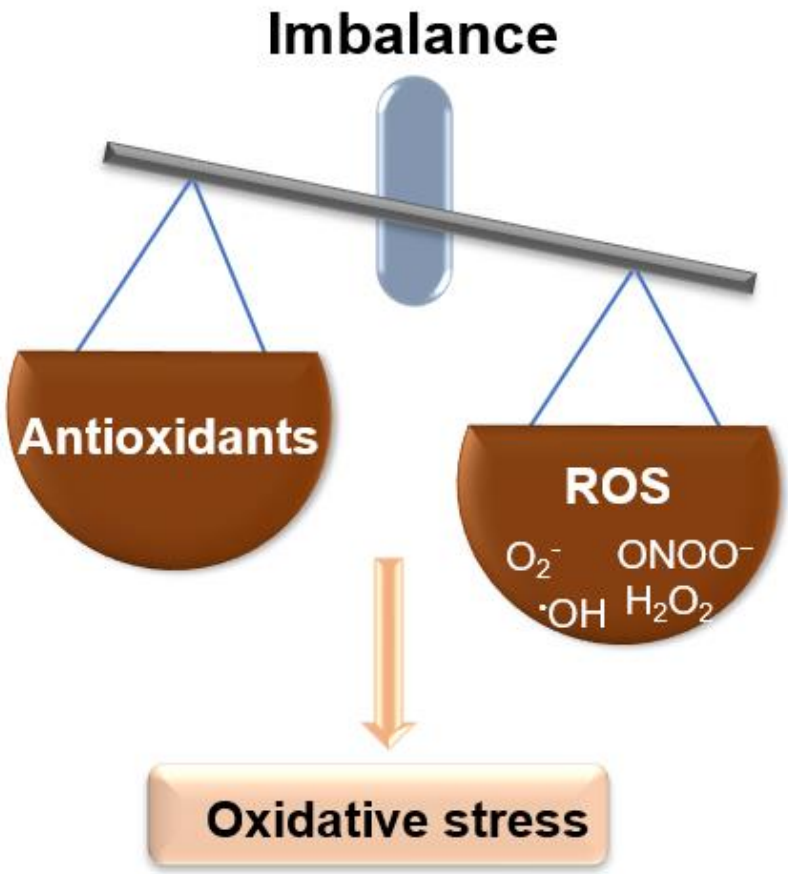

Fig. 7. Oxidative stress (Liu et al., 2017).

1.3.2. Oxidative stress and lipid peroxidation

Low ROS levels contribute to various normal biological processes, such as immune-mediated defence against pathogenic microorganisms and facilitation of intracellular signal

transduction (Burdon et al., 1996; Moldogazieva et al., 2018). In contrast, excessive ROS can result in the damage of DNA, protein oxidization, and lipid peroxidation, which all can change the normal functions of cells (Venardos et al., 2007). Among these, lipid peroxidation caused by oxidative stress is a process in which the free radicals (for example, the hydroxyl free radical, peroxide free radical and oxygen free radical) remove electrons from lipids and then generate membrane damaging reactive intermediates (Que et al., 2018; Su et al., 2019). Such reactive intermediates have been revealed to destroy DNA, proteins, and enzyme activity and activate signalling pathways initiating cell death (Łuczaj et al., 2017) (**Fig. 8**). In both cell culture and animal models, lipid peroxidation has been shown to damage cellular membranes and impair organelle functions (Hauck and Bernlohr, 2016).

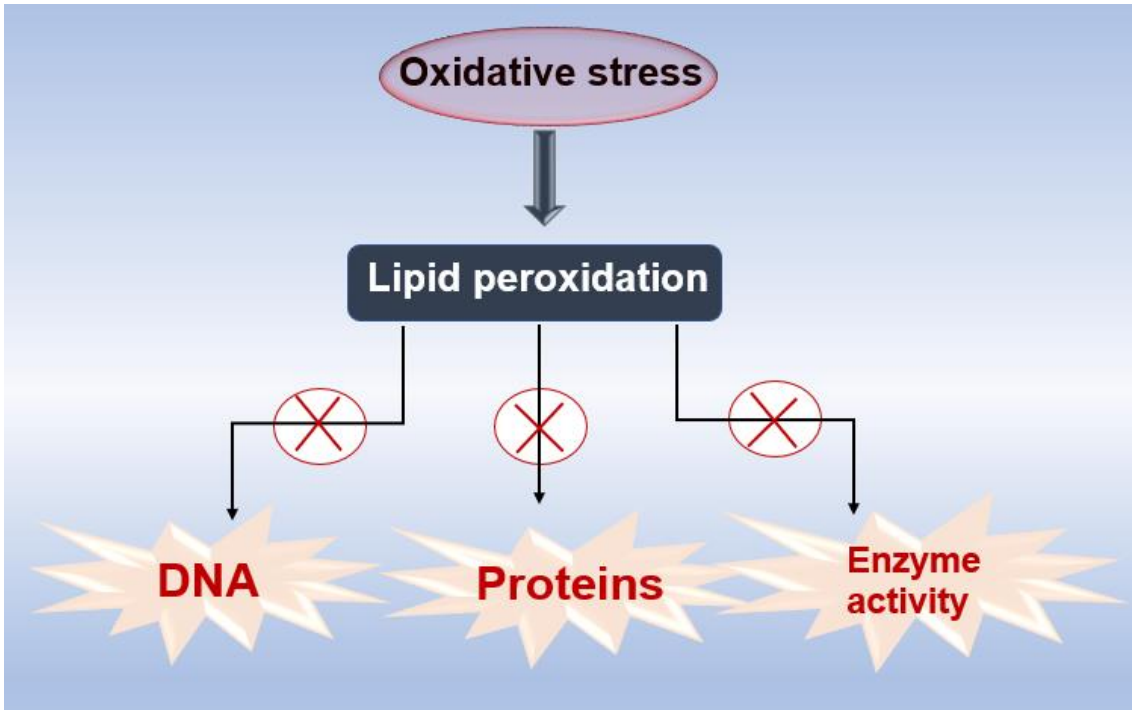

Fig. 8. Oxidative stress and lipid peroxidation (Rezayian et al., 2019).

1.3.3. Oxidative stress and mitochondrial dysfunction

About 90 percent of the ROS generated in mitochondria are derived from electron leakage from respiratory chains at mitochondrial complex I and complex III (Andreyev et al., 2015; Boengler et al., 2017; Kanaan and Harper, 2017; Kato et al., 2017; Makrecka-Kuka et al., 2020). Generally, more than 90 percent of the oxygen is used for the production of ATP in mitochondria, while approximately 2 percent of oxygen is converted into ROS as electron transport chain (ETC) by-products (Perrin et al., 2012). It has been found that ROS generation in the mitochondria results in the formation of lipid peroxides, which, in turn, induce subsequent damage to mitochondria. The mitochondria are essential organelles for cell survival and function. Mitochondrial damage contributes to a variety of pathologies, including accumulated damage to their DNA (mitochondrial DNA), decreased mitochondrial respiration and reduced ATP production, permeability transition pore (PTP) opening, further inducing the permeability transition of mitochondria (Sverdlov et al., 2016). These changes result in mitochondrial decomposition, membrane potential depolarization, excessive contraction of cardiomyocytes, and even cell death via activating apoptotic signal transduction (Aon et al., 2006; Dalmasso et al., 2017; Goh et al., 2016; Rannikko et al., 2013; Rasola and Bernardi, 2007; Vieira et al., 2017).

Furthermore, with PTP, ROS can leave the mitochondria to facilitate further release of ROS from adjacent mitochondria together with other sources through a process called ROS-induced ROS release (Ago et al., 2010) (**Fig. 9**). Studies in animal and human models have shown that oxidative stress can affect various cellular phenotypes, including cardiomyocyte hypertrophy, senescence, and cell proliferation (San Martín et al., 2007; Wu et al., 2019). As such, reducing mitochondrial ROS accumulation and avoiding mitochondrial oxidative damage may help prevent heart disease.

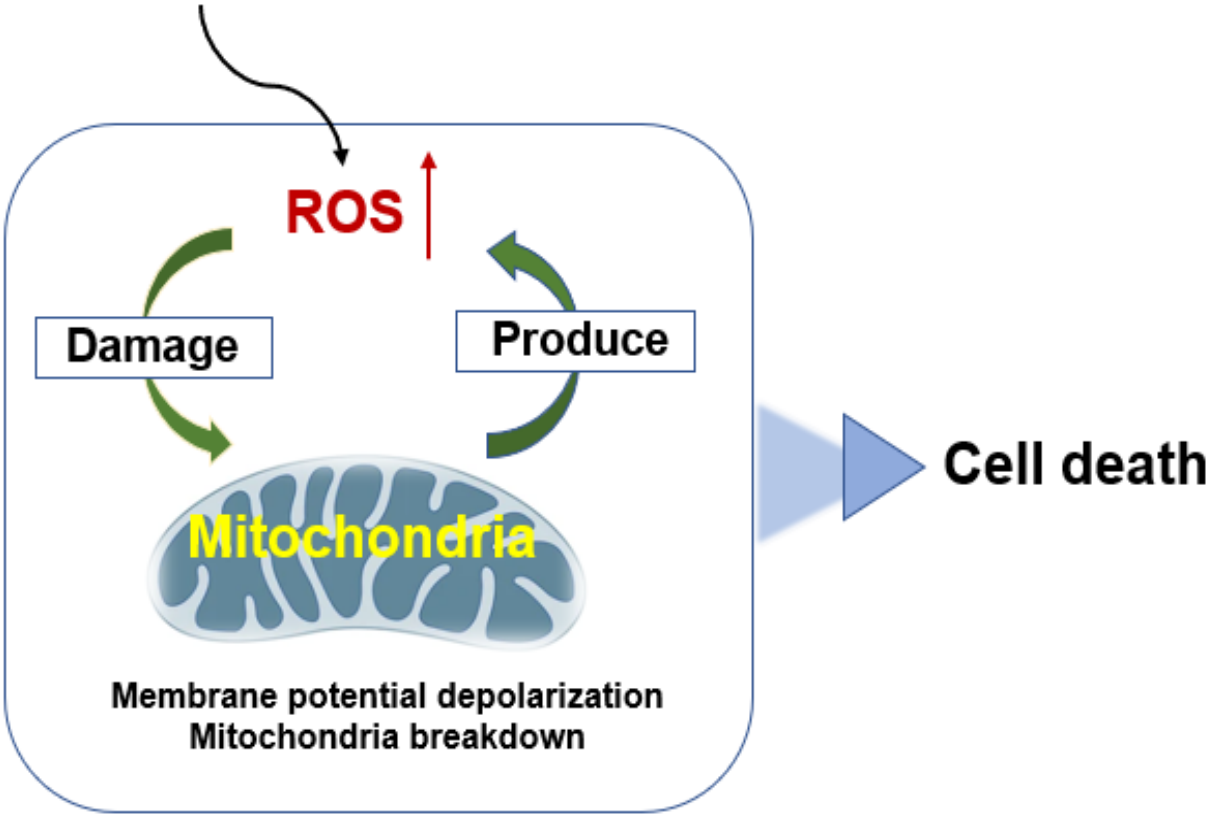

Fig. 9. Oxidative stress and mitochondrial dysfunction (Topf et al., 2016).

1.3.4. Lysine acetylation and histone acetylation

Lysine acetylation refers to an acetyl group (CH_3CO) addition to organic compounds containing lysine residues through enzymatic acetylation with acetyltransferase or the non-enzymatic chemical modification with acetyl-CoA (Hebert et al., 2013; Hirschey et al., 2010; Zhang et al., 2009). Acetylation of lysine regulates the biophysical performances of these modified proteins, which includes changes in protein stability (Shaw et al., 2008; Xiong and Guan, 2012). Deacetylases can reverse this modification. Similarly, the balance between histone deacetylase (HDAC) and histone acetyltransferase (HAT) is employed to control the cellular levels of histone acetylation (**Fig. 10**). Histone acetylation affects local chromatin architecture and promotes gene expression in the affected region. However, deacetylation of histone catalyzed by HDAC induces chromatin condensation and inhibits gene expression (Bannister and Kouzarides, 2011; Cedar and Bergman, 2009; Kuschman et al., 2021). In humans, eighteen HDACs have been divided into 4 different groups, namely, Class I HDACs (Zn^{2+} dependent HDACs, containing HDAC1, 2, 3, along with HDAC 8), Class II HDACs (related to yeast HDA1, including HDAC 4, 5, 6, 7, 9, and HDAC 10), Class IV HDACs (HDAC11), and Class III HDACs (also called Sirtuins, containing SIRT1, 2, 3, 4, 5, 6, and 7). Among these, Class III HDACs need the NAD+ to act as a reactant in deacetylation reactions (Mobley et al., 2017; Seto and Yoshida, 2014).

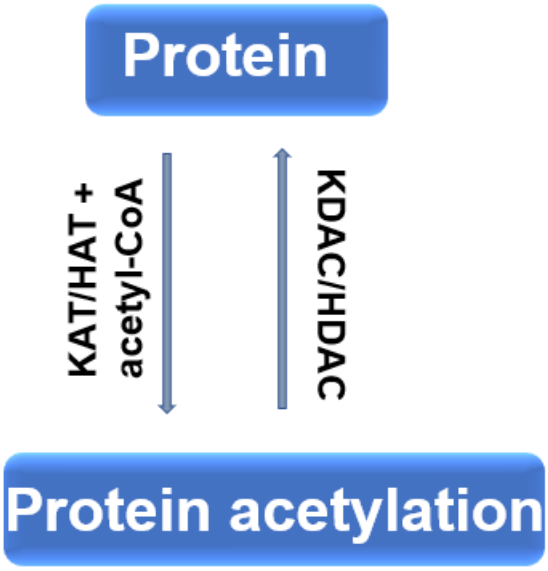

Fig. 10. Lysine acetylation and histone acetylation (Christensen et al., 2019).

1.3.5. Protein acetylation, histone acetylation-related enzymes and heart function.

A recent study found that the pathophysiology of heart dysfunction is associated with excessive protein acetylation (Lkhagva et al., 2018). The calcium-binding protein cardiac troponin I (cTnI) is a crucial protein regulating cardiac contraction, and its reduction is related to diastolic dysfunction. It has been shown that cTnI acetyl-mimetic mutations reduce cTNI-mediated cardiac contractility and up-regulate relaxation speed in cultured rat cardiomyocytes (Lin et al., 2020). In the heart failure with preserved ejection fraction (HFpEF) animal model, inhibition of protein acetylation improved diastolic function (Wallner et al., 2020).

The activation of class I HDACs critically affects the cardiac structure and electrical remodelling in heart failure. The inhibition of Class I HDAC has been confirmed to increase the expression of superoxide dismutase-2 (SOD2) and catalase (CAT), reduce ROS, downregulate inflammatory cytokines, and decrease cardiac fibrosis and hypertrophy (Francois et al., 2021b). Besides, both the myocardial infarction and ischemic reperfusion (IR) model suggested that inhibition of Class I HDAC blunts hypertrophy and preserves systolic functions (Aune et al., 2014; Gallo et al., 2008; Morales et al., 2016; Nural-Guvener et al., 2014). Moreover, mitochondrial HDAC1 inhibition by LL-66, a selected inhibitor of Class I HDACs, induced a protective effect on reperfusion injury. The treatment of LL-66 can also decrease ROS generation, contributes to increased cardiomyocyte viability and improved left ventricular function. The unspecific inhibition of all HDACs with a general inhibitor of HDACs, called suberoylanilide hydroxamic acid (SAHA), improved cardiomyocyte survival following I/R injury (Yang et al., 2019).

1.3.6. Protein acetylation, histone acetylation-related enzymes and cardiac ageing

Lysine acetylation was observed to be increased in ageing rat hearts (Francois et al., 2021a; Yeo et al., 2020). Besides, it has been shown that the dysregulation of class I HDACs are involved in cardiac senescence (Pinkerneil et al., 2016; Singh et al., 2018). The benefits of inhibiting Class I HDACs in cardiac ageing through regulating anti-inflammatory and anti-oxidative mechanisms have also been fully recognized (Francois et al., 2021a).

Furthermore, Sirt1 (a member of the Class III HDACs) has targets involved in signalling pathways related to cardiac ageing, including FOXO1, p53, eNOS and JNK (Orimo et al., 2009; Ota et al., 2007). Among them, p53 is involved in DNA damage response, cell cycle regulation, and cardiomyocyte homeostasis (Levine, 2019). It has been revealed that following DNA damage, activated Sirt1 causes deacetylation and inhibition of p53, thus preventing cardiac senescence (Kang et al., 2009). It was also found that the activation of Sirt1 was diminished with age, leading to upregulation of p53 and cellular senescence (Sasaki et al., 2006). Moreover, cardiac overexpression of Sirt1 attenuated cardiac ageing with improved systolic function, reduced hypertrophy, and lower expression of senescence markers (Alcendor et al., 2007).

1.3 7. Characteristics of senescent cells

Cellular senescence prevents the replication of cells harbouring damaged DNA, which is a critical mechanism limiting tissue damage and tumorigenesis. In physiological conditions, senescent cells can be removed by the immune system; however, senescent cells accumulate in tissues as we age. The long-standing senescent cells that exist in the organs cause ageing and diseases related to age.

There are three types of senescence, namely oncogene-induced senescence, replicative senescence, and stress-induced senescence. Among these, stress-induced cell senescence is characterized by pro-inflammatory senescence-associated secretory phenotype, upregulated senescence-associated β-galactosidase (SA-β-gal), morphological and metabolic changes, altered gene expression, more significant damage of DNA, and mitochondria dysfunction (Collado et al., 2007) (**Fig. 11**). The senescent cardiomyocytes show the hallmarks of senescence-associated secretory phenotype and contribute to cardiac remodelling and failure. A recent study revealed that H_2S could attenuate ageing by inhibiting cellular senescence (Perridon et al., 2016). However, the regulatory roles of H_2S against the cardiac senescence induced by lipid overload are not clear yet.

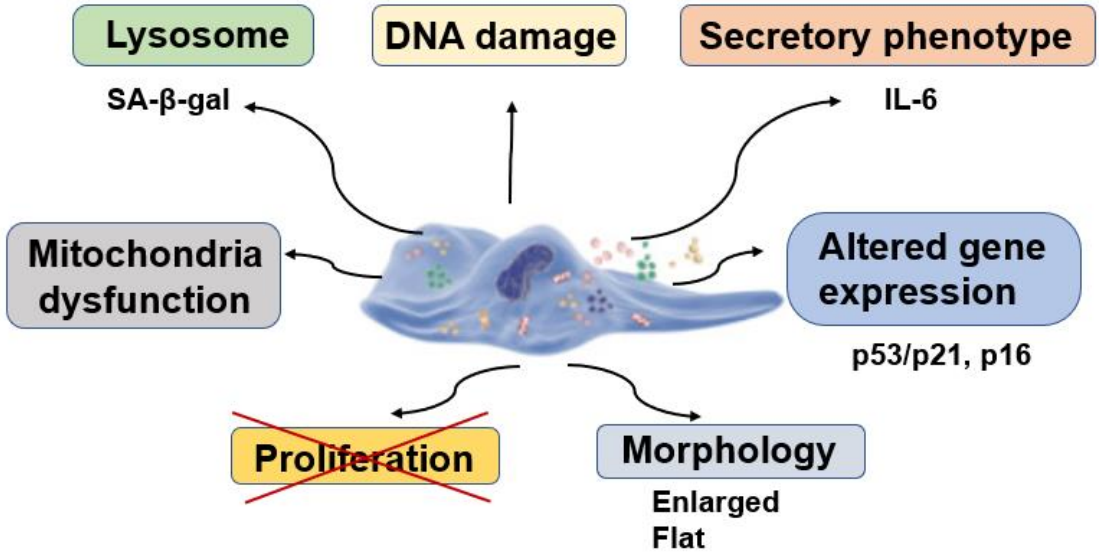

Fig. 11. The feature of senescence (Shimizu and Minamino, 2019).

2. Hypothesis and objectives

This project hypothesized that H_2S is essential for maintaining lipid homeostasis and preventing cellular senescence in heart cells under lipid overload. To this end, by utilizing an *in vivo* animal model and an *in vitro* cell culture model, three objectives were proposed: 1) Explore the effects of H_2S on lipid overload-induced lipotoxicity; 2) Investigate the *in vitro* role of H_2S in lipid overload-induced cardiomyocyte cell senescence; 3) Study the *in vivo* role of an H_2S-generating gene CSE in lipid homeostasis and cardiac ageing. The goal of this project was to provide evidence to support H_2S's role in preventing and treating obesity- and age-related heart diseases.

3. Materials and Methods

3.1. Cell culture

Rat cardiomyocytes (H9C2, American Type Culture Collection, Manassas, VA) were cultured in Dulbecco's modified Eagle medium containing streptomycin (100 mg/ mL), penicillin (100 U/ mL) and 10% heat-inactivated fetal bovine serum in a 5% CO_2 humidified atmosphere at 37°C. Dulbecco's PBS was utilized to wash cells, and a new medium was added every other day. H9C2 cells were cultured to 80% confluence to avoid cell differentiation. H9C2 cells with a confluence of 75-80% between passages 8 and12 were employed in a variety of treatments. For the treatment with FFAs, the cells were exposed to Dulbecco's modified Eagle medium supplemented with a chemically defined lipid mixture

(5 µl/ml) containing Tween-80 (2.2 mg/ml), cholesterol (0.22 mg/ml), the non-animal derived fatty acid arachidonic acid (2 µg/ml) and 10 µg/ml each of myristic, linolenic, linoleic, stearic, palmitic and oleic acids. The lipid mixture also contained 100 mg/ml of pluronic F-68 to reduce foaming (Sigma-Aldrich, Oakville, ON) and tocopherol acetate (Vitamin E, 70 µg/ml).

3.2. Western blotting

The mouse heart tissues or H9C2 cells were harvested, and then they were solubilized in Tris/EDTA/Sucrose buffer in the presence of protease inhibitors (Sigma-Aldrich). The SDS-PAGE protein loading buffer (a ready-to-use 5× solution, containing 10% SDS, 500 mM DTT, 500 mM Tris-HCL pH 6.8, 0.05% bromophenol blue dye, 2-β-mercaptoethanol and 50% glycerol) was mixed with equal amounts of protein (50 µg/well), and the samples were boiled for 5 minutes prior to loading onto a 12.5% polyacrylamide SDS gel. These gels were then electrophoresed, and the resolved proteins were transferred to PVDF membranes (Pall Corporation, Pensacola, FL). Phospho-buffered saline solution (PBS) supplementing with 0.1% Tween 20 and 5% skim milk was utilized to block membranes at room temperature for 2 hours and then incubated at 4 ℃temperature overnight with the primary antibody in blocking buffer. After the incubation with the primary antibody, the membranes were washed 3 times with PBS-T (phospho-buffered saline and 0.1% tween-20) solution and subsequently incubated with horseradish peroxidase-bound anti-mouse or anti-rabbit secondary antibody (1:5000, Sigma Aldrich) 90 minutes at room temperature. Finally, the chemiluminescence signal was detected using an ECL reagent (GE Healthcare, Amersham, UK). The primary

antibodies dilutions were as below: long-chain acyl-CoA synthetase 1 (ACSL1, 1:1000, Cell Signaling Technology, Danvers, MA), CSE (1:1000, Abnova, Taipei), GAPDH (1:1000, Sigma-Aldrich), acetylated lysine (1:1000, Cell Signaling Technology), cytoplasmic acetyl-CoA synthetase 1 (AceCS1, 1:1000, Cell Signaling Technology), ATP-citrate lyase (ATP-CL, 1:1000, Cell Signaling Technology). Digital copies of the western blots were made by scanning the film, and the relative intensity was calculated via density analysis using ImageJ software. The outcomes were described as the ratio of target protein expression to GAPDH expression.

3.3. Real-time PCR

For total RNA isolation, 100 mg of heart tissue or cells was dissolved with the Trizol reagent (1 ml) (Invitrogen, Carlsbad, CA). After lysis, chloroform (200 μl) was added to samples, and the centrifugation of such samples was conducted at 4 °C temperature for fifteen minutes at 12,000 × *g*. The RNA in the upper aqueous phase was precipitated by incubating with the same volume of isopropanol at ambient temperature for ten minutes and then centrifuged at 4℃temperature for fifteen minutes at 12000 × g. RNA pellets were washed once with 70% ethanol, after which the RNA was solubilized in RNase-free water (40 μl) (Chen et al., 2014; Ma and Schultz, 2008; Yang et al., 2018). The ReverAid™ First Strand cDNA Synthesis Kit was employed to prepare cDNA from the RNA preparations (Thermo Fisher Scientific, Waltham, MA). The primer sequences used in PCR are reflected in Table 1. All of the samples were run in triplicates, and the relative expression of each gene was

calculated using $2^{-\Delta\Delta CT}$ and expressed as a percentage over the control samples (Lee et al., 2016).

Table 1: Primers sets utilized for the analysis of real-time PCR in H9C2 cells.

GAPDH (NM_002046.5)	5'-GCGGGGCTCTCCAGAACATCAT-3' (forward) 5'-CCAGCCCCAGCGTCAAAGGTG-3' (reverse)
ACOX1 (NM_004035.7)	5'-CCCGAAAGCCTAACCGAAGCATA-3' (forward) 5'-CATCATAGCGGCCAAGCACAGAG-3' (reverse)
ApoB (NM_000384.3)	5'-TGACCGGGGACACCAGATTAGA-3' (forward) 5'-CAGGCGACCAGTGGGCGAGGAT-3' (reverse)
CD36 (NM_000088.4)	5′- GAGAGAACTGTTATGGGGCTAT-3′ (forward) 5′-TTCAACTGGAGAGGCAAAGG -3′ (reverse)
PPARα (NM_001001928.3)	5'-TGCCCCCTCTCCCCACTCG-3' (forward) 5'-AGCCCTTGCAGCCTTCACACG-3' (reverse)
HDAC1 (NM_008228.2)	5′-TGGGGCTGGCAAAGGCAAGT-3′ (forward) 5′-GACCACTGCACTAGGCTGGAACA-3′ (reverse)
HDAC2 (NM_ 008229.2)	5′-CGTACAGTCAAGGAGGCGGCAA-3′ (forward) 5′-TGAGGCTTCATGGGATGACCCTGG-3′ (reverse)
HDAC3 (NM_010411.2)	5′-ACGTGCATCGTGCTCCAGTGT-3′ (forward) 5′-AGTGTAGCCACCACCTCCCAGT-3′ (reverse)

SIRT1 (NM_001372090)	5′-TGCTGGCCTAATAGAGTGGCA-3′ (forward) 5′-CTCAGCGCCATGGAAAATGT-3′ (reverse)
ANF (M27498.1)	5’-AGCGGGGGCGGCACTTA-3’ (forward) 5’-GGGCTCCAATCCTGTCAATCCTAC-3’(reverse)
BNP (M25297.1)	5’-CCTAGCCAGTCTCCAGAACAATCC-3’ (forward) 5’-CTAAAACAACCTCAGCCCGTCACA-3’ (reverse)

3.4. Cell viability assay

As previously described, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used to evaluate the viability of cells (Chatzianastasiou et al., 2016). In short, 10,000 cells per well were seeded into 96-well plates. When the cell density reached around 50% confluence, cells were then cultured with various concentrations of a lipid mixture (0–200 μM) without or with NaHS in darkness at 37 °C for 1-3 days. After removing the medium, 10 μl of a 0.5 mg/ml MTT solution was added to each well, and the plates were cultured in darkness at 37 °C for four hours. Dimethyl sulfoxide (100 μl) was then added to dissolve the MTT formazan, and the absorbance of each well at 570 nm measured using an FLUO OPTIMA microplate spectrophotometer (BMG LABtech, Germany). The absorbance of untreated cells was regarded to be 100 percent viable. An average of six wells was used per treatment, and all experiments were independently conducted 3 times or more.

3.5. Cell apoptosis analysis

Cell apoptosis was detected by measuring cellular caspase 3/7 activity. After various treatments, the media was removed from the cells, and CellEvent™ Caspase-3/7 Green Detection Reagent (Diluted into PBS with 5% FBS to a final concentration of 4 μM, Caspase 3/7) (Thermo Fisher Scientific, Ottawa, Canada) was added to the cells. Then cells were cultured in a 35 mm culture dish at 37°C in darkness for thirty minutes. The stained cells were immediately imaged on an Olympus CX71 fluorescent microscope (Olympus, Tokyo, Japan) after washing, and the activated cells of caspase 3/7 were counted utilizing the Image J 1.43 software.

3.6. Senescence-associated β-galactosidase staining

An SA-β-gal staining kit (Cell Signaling Technology, Beverly, MA) was used for cellular β-Galactosidase staining. Briefly, the cells were placed at room temperature and fixed with fixation solution for fifteen minutes. Subsequently, cells were incubated at 37 ℃until the β-Gal staining can be seen in the staining solution. An Olympus CX71 fluorescent microscope was used to take these images. A total of 400 cells were counted in each culture dish, and the percentage of SA-β-gal positive cells was then counted and calculated.

3.7. Measurement of oxidative stress

DCF-DA (2′, 7′-dichlorodihydrofluorescein diacetate) can penetrate cell membranes and then be deacetylated to DCFH (2,7-dichlorodihydrofluorescein) via cellular esterase. This non-fluorescent product can then be transformed by reactive oxygen species (ROS) into dichlorofluorescein (DCF), which is highly fluorescent. Using this approach, after a variety of

cell treatments, ROS levels in the cells were detected using 1 μg/ml DCFDA (Invitrogen, Carlsbad, CA) in a wet environment at 37°C in darkness for fifteen minutes. Subsequently, the cells were gently washed twice with PBS. The fluorescence intensity was then measured using a fluorescence microscope (Wang et al., 2021).

3.8. Mitochondrial function

JC-1, a mitochondrial-specific cationic dye, was employed to detect changes in mitochondrial membrane potential ψ_m (Abcam Inc., Toronto). JC-1 is a lipophilic cation, which can enter into mitochondria selectively. After a variety of treatments, the H9C2 cells were cultured in JC1 (10 μM) in the dark for ten minutes at 37°C, and the cells were then washed twice with PBS. Fluorescence microscopy was subsequently employed to capture and analyze the fluorescent signal. The red emission indicates that JC-1 accumulates in the mitochondria in a membrane potential-dependent fashion. At the same time, the green fluorescence exhibits the monomeric morphology of JC-1 in the cytoplasm after the depolarization of the mitochondrial membrane. As a result, the depolarization of mitochondria is manifested as a reduction in the ratio of green/red fluorescence strength as quantified by Image J software (Wang et al., 2021).

3.9. Measurement of lipid peroxidation

Lipid peroxidation was measured by staining the cells with BODIPY 581/591 C11 (Thermo Fisher Scientific, Ottawa, ON). After various treatments, the cells were washed

twice with PBS and labelled with 5 μM BODIPY 581/591 C11 at 37 °C for ten mins. After the cells were washed twice with PBS, the green and red fluorescence were then observed with a fluorescent microscope. The BODIPY 581/591 C11 value was calculated as the ratio of the green fluorescence (which indicates oxidized probe) to total fluorescence (green + red), which is the total fluorescence of both the reduced and oxidized probes (Wang et al., 2021).

3.10. Oil Red O staining

Oil Red O staining was utilized for quantifying the accumulation of lipids in cells (Ali et al., 2020; Sun et al., 2015; Yang et al., 2018). After different treatments, the H9C2 cells were fixed, and the cells were then stained with Oil Red O using the Lipid Accumulation assay kit (Abcam). Under light microscopy, the stained lipids are red and can be quantified using image J software (Olympus, Tokyo, Japan).

3.11. Animal feeding

CSE knockout (KO) mice and wild-type (WT) mice were all fed under a standard rat chow diet with water and food before feeding on a high-fat diet (HFD). In this present work, eight-week-old male mice were fed either HFD containing 60% of kcal/fat (Research Diets, New Brunswick, NJ) or the control diet including 10% of kcal/fat (Research Diets, New Brunswick, NJ) for twelve weeks. At the end of this experiment, the mice were sacrificed, and the heart tissues were removed and frozen immediately at -80 ℃ for the further studies. All

the animal studies were implemented in the light of the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and authorized by the Animal Care Committee of Laurentian University, Ontario, Canada.

3.12. Statistical analysis

All experiments were conducted 3 times or more. The data points are pictured to display the means ± standard error (SEM) for each experiment. A one-way analysis of variance (ANOVA) or Student's *t*-tests (SPSS19.0) were performed for statistical comparisons between data sets using Tukey's post-hoc test. A p-value of < 0.05 was considered statistically significant.

4. Results

4.1. H_2S protects cardiac cells from lipid overload-inhibited cell viability

To determine the influence of excess lipid *in vitro*, the H9C2 cells were cultured in 5 μl/ml lipid mix without or with NaHS of 30 μM for 1-3 days. The role of lipid overload in the viability of cardiac cells was detected with MTT assay. As shown in **Fig. 12A**, lipid mix inhibited cardiac cell viability with a time-dependent mode compared with the control group ($p < 0.05$). The co-incubation with H_2S donor, NaHS, alleviated lipid overload-inhibited cell viability in H9C2 cells. Besides, Caspase 3/7 Green dye was utilized to label the cells for

detecting the effects of lipid overload on apoptosis. The data revealed that lipid overload had little effect on cell apoptosis (**Fig. 12B**).

Under the stress of lipid overload, hypertrophy is the typical pathological response of cardiomyocytes (Battiprolu et al., 2012; Monji et al., 2013). Thus, we explored the effect of H_2S treatment on cardiac remodelling from the perspective of brain natriuretic peptide (BNP) and the atrial natriuretic peptide (ANF) mRNA expression. These two main cardiac secretion products are recognized as markers of cardiac hypertrophy. Real-time PCR results demonstrated lipid overload significantly induced the transcription of the genes for both ANF and BNP, while the addition of NaHS restored their expressions (**Fig. 12C**, **D**). Furthermore, we evaluated CSE gene expression, the primary enzyme generating H_2S in the cardiovascular system (Patel et al., 2009; Yang et al., 2005). As shown in **Fig. 12E**, CSE protein expression did not exhibit a significant change in the lipid overloaded cells compared to the control group.

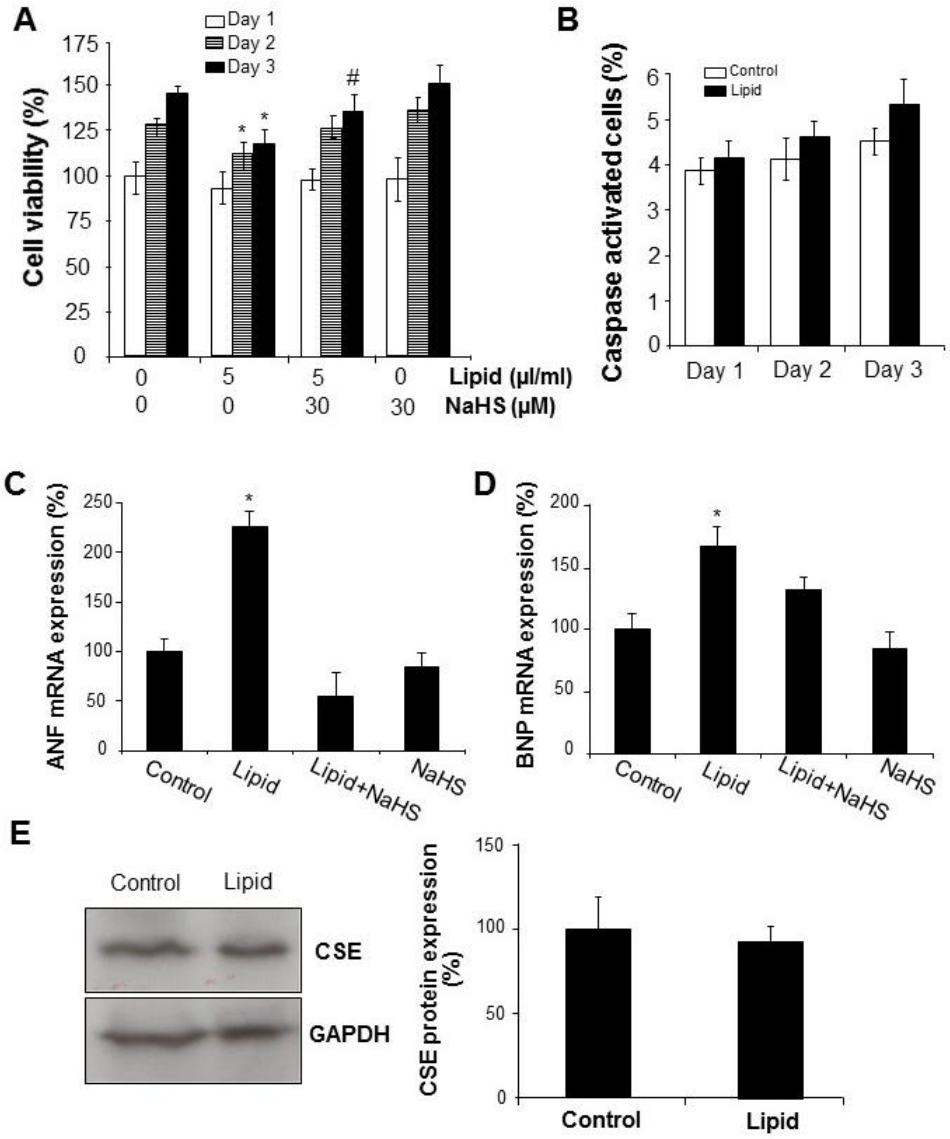

Fig. 12. H_2S protects cardiac cells from lipid overload-inhibited cell viability. ***A***, NaHS can reduce the cell viability inhibited by lipid overload in the H9C2 cells. Without or with NaHS of 30 μM, the cells were cultured with 5 μl/ml lipid mix for 1-3 days, and then the cell viability was detected by MTT. n=5. *, $p<0.05$ versus control group at the same day; #, $p<0.05$ versus lipid group at the same day. ***B***, Lipid overload had little effect on cell apoptosis. After incubating H9C2 cells with 30 μM NaHS and/or 5 μl/ml lipid mix for 1-3 days, cells were labelled with Caspase 3/7 Green to detect apoptosis. n =3. ***C*** and ***D***, NaHS

reversed lipid overload-induced mRNA expressions of ANF and BNP. Cells were incubated by 30 μM NaHS and/or 5 μl/ml lipid mix for 3 days. RNA was extracted, and mRNA expression was tested via utilizing real-time PCR. n=3. *, $p<0.05$ versus all other groups. ***E***, Lipid overload did not change CSE expression. A lipid mix of 5 μl/ml was utilized to incubate the cells for three days, and the expression of CSE was analyzed by western blotting. n=4

4.2. H_2S inhibits lipid accumulation in cardiac cells by regulating lipid metabolism-related genes

To investigate whether H_2S affects lipid accumulation in H9C2 cells, the lipids were detected with Oil Red O staining. Compared with the control group, exposure of H9C2 cells to the lipid mixture caused a marked increase in intracellular lipids, and exogenous H_2S treatment significantly reduced lipid accumulation. NaHS treatment alone did not affect cellular lipid levels (**Fig. 13A**). Furthermore, with the aim of exploring the underlying mechanism of H_2S protection from lipid accumulation, real-time PCR and western blotting were employed to analyze the expression of genes associated with lipid metabolism. AceCS1 and ATP-CL are two representative types of enzymes participating in lipid biogenesis, which catalyze acetyl-CoA formation in the cytosol, contributing to de novo fatty acid synthesis. The AceCS1 and ATP-CL expression levels were evaluated to explore whether H_2S would influence de novo fatty acid synthesis in the cardiac cells. As expected, lipid overload markedly enhanced the protein expression level of both ATP-CL and AceCS1 (**Fig. 13B**). Compared to the lipid overload group, H_2S treatment blocked the expressions of ATP-CL and

AceCS1. ACSL1 is associated with catalyzing the ligation of fatty acids to coenzyme A to form fatty acyl-coenzyme A. As illustrated in **Fig. 13B**, protein expression of ACSL1 did not change significantly by either lipid overload or NaHS incubation.

Transcription factor peroxisomal proliferator-activated receptor α (PPARα) effectively activates fatty acid β-oxidation-related genes, including the rate-limiting enzyme acyl-CoA oxidase 1 (ACOX1). As reflected in **Fig. 13C**, the mRNA levels of ACOX1 and PPARα were remarkably enhanced in the lipid overload group compared with the control group, while NaHS treatment attenuated PPARα and ACOX1 expressions in the presence of lipid mix. These data indicate that H_2S can decrease fatty acid β-oxidation induced by lipid overload via inhibiting PPARα and ACOX1.

The apolipoprotein B (ApoB) mRNA expression, responsible for the secretion of lipid (Bjorkegren et al., 2001; Nielsen et al., 2002a; Nielsen et al., 2002b), was remarkably downregulated by lipid mix (**Fig. 13C**). Further co-incubation with H_2S reversed the inhibitory role of lipid overload-induced ApoB mRNA expression. It is speculated that H_2S may partially inhibit myocardial lipid accumulation by promoting ApoB-related lipid secretion. Similarly, lipid overload also markedly decreased the mRNA expression level of CD36, a predominantly membrane protein involved in the uptake of fatty acid into cardiac cells (Li et al., 2019; Nakatani et al., 2019). Exogenously applied NaHS partially reversed the inhibitory role of lipid mixture on CD36 expression (**Fig. 13C**). Our data also suggest that

H_2S may promote lipid uptake that was decreased by lipid overload. Based on all these data, H_2S can inhibit intracellular lipid accumulation in cardiac cells by regulating lipid metabolism-related genes.

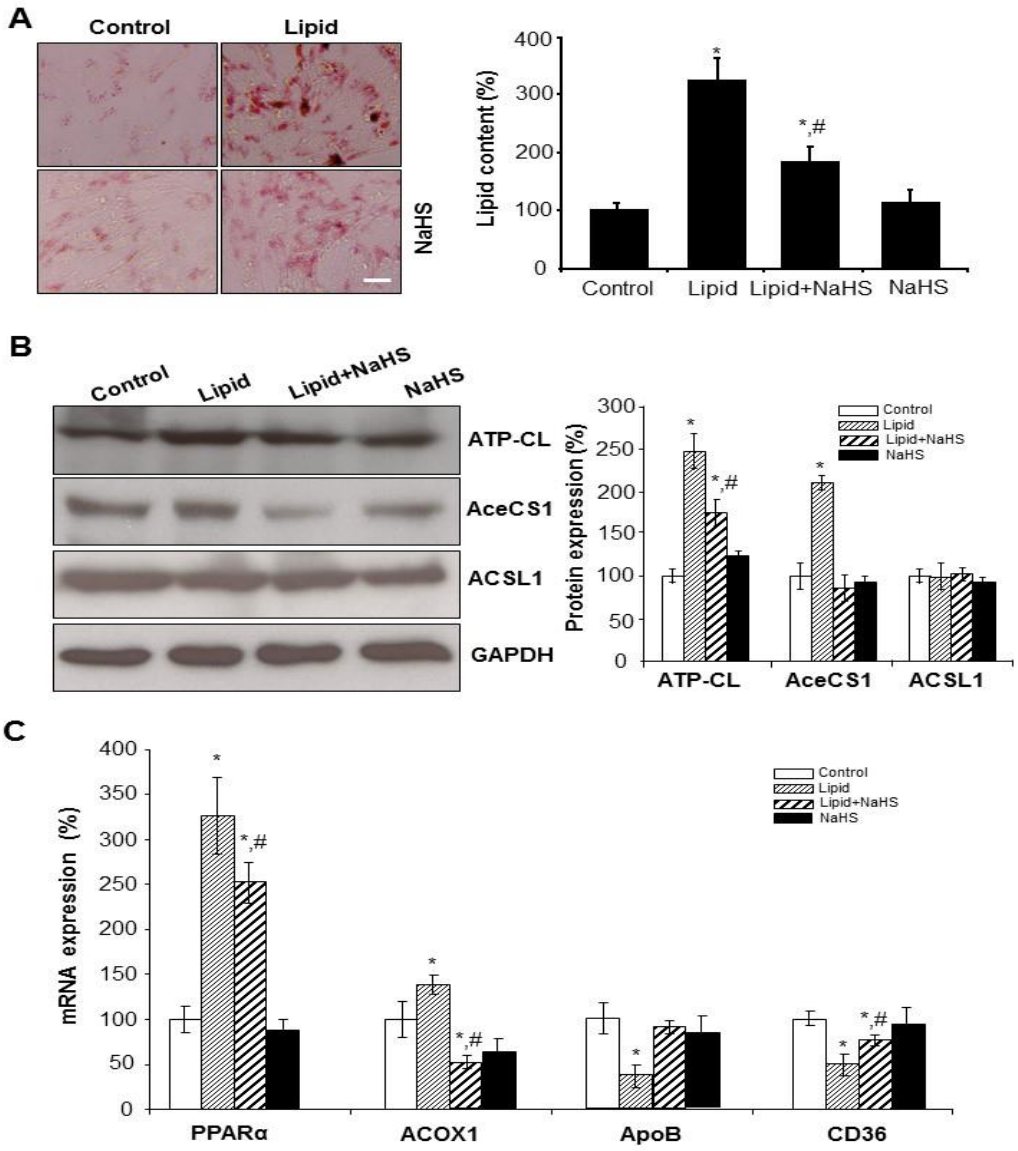

Fig. 13. H_2S inhibits lipid accumulation in cardiac cells by regulating lipid metabolism-related genes. After incubating the cells with a 5 μl/ml lipid mixture in the presence or absence of NaHS (30 μM) for three days, the lipid accumulation was analyzed via Oil Red O

staining (***A***). Scale bar in ***A***: 20 μm. The expression of lipid metabolism-related genes was further explored with western blotting (B) and real-time PCR (***C***). n=3. *, $p < 0.05$ vs. control cells; #, $p < 0.05$ vs. lipid group.

4.3. H_2S suppresses lipid overload-induced oxidative stress, mitochondrial dysfunction, and lipid peroxidation

Emerging evidence indicates that excessive lipid accumulation causes cardiovascular dysfunction by inducing oxidative stress (Karam et al., 2017). Thus, we investigated the influences of NaHS treatment and lipid overload on the intracellular levels of ROS. Higher intracellular ROS levels were observed in response to lipid mix administration compared to the control group, while NaHS treatment could effectively reduce lipid-induced intracellular ROS levels (**Fig. 14A**). Besides, mitochondria are the lipid metabolism center, and mitochondrial injury is closely associated with abnormal lipid metabolism (Chen et al., 2020). Lipid-induced mitochondrial structural changes were detected as measured by reductions in mitochondrial membrane potential (ΔΨm) by staining the cells with JC1 dye. As shown in **Fig. 14B**, in comparison with untreated cells, an evident reduction in ΔΨm was found in cardiomyocytes incubated with lipid mix, pointing to the impaired mitochondrial function. In contrast, the lipid mix-induced decrease of ΔΨm was reversed by NaHS co-incubation with lipids in the cardiac cells. The obtained results suggest that H_2S suppresses lipid overload-induced mitochondrial dysfunction.

In addition, lipid peroxidation is the oxidative degradation process of lipids, including membrane lipids. During this process, oxygen reacts with unsaturated lipids to produce various oxidation products, such as lipid hydroperoxides (LOOH), malondialdehyde (MDA), which can destroy DNA, proteins, and enzyme activity (Łuczaj et al., 2017). It was then observed that lipid incubation caused more lipid peroxidation, as shown by a higher proportion of green to red fluorescence when C11 Bodipy stained cells. After supplementation with NaHS, the green/red ratio returned to levels seen for untreated control cells (**Fig. 14C**). In conclusion, H_2S appears able to protect H9C2 cells from lipid peroxidation, mitochondrial dysfunction and oxidative stress induced by lipid overload.

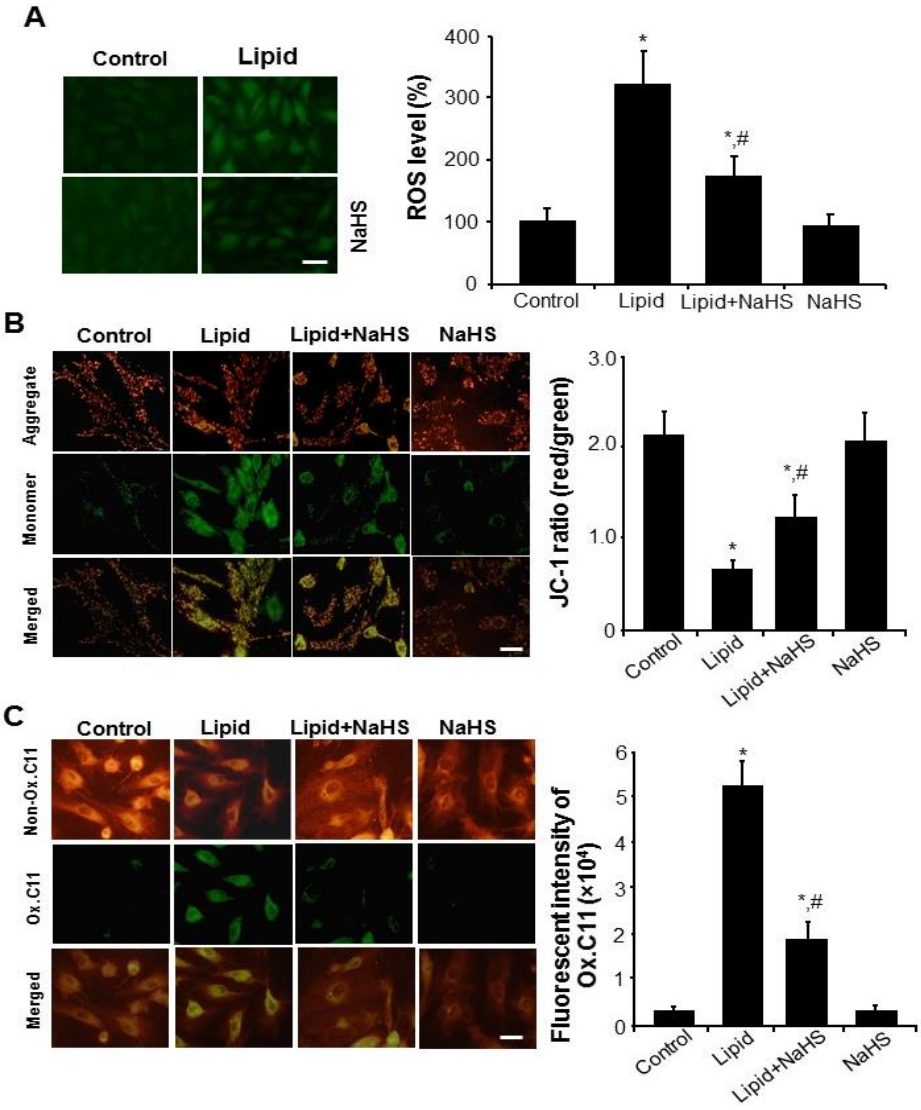

Fig. 14. H_2S suppresses lipid overload-induced oxidative stress, mitochondrial dysfunction, and lipid peroxidation. After incubating H9C2 cells with 5 μl/ml lipid mix without or with NaHS of 30 μM for three days, the cells were subjected to detection of oxidative stress by staining the cells with H_2DCFDA (***A***), the mitochondrial membrane potential via staining cells with JC1 dye (***B***), and lipid peroxidation by staining the cells with BODIPY 581/591 C11 (***C***). Scale bar: 20 μm. *, $p < 0.05$ vs. control cells; #, $p < 0.05$ vs. lipid group.

4.4. H_2S protects H9C2 cells from lipid overload-induced cellular senescence

To analyze the effect of H_2S on cardiac cell senescence, we treated H9C2 cells with a lipid mixture (5 µl/ml) without or with 30 µM NaHS for three days. Cardiac cells' senescence was quantified with SA-β-gal staining. The SA-β-gal staining confirmed that H9C2 cell senescence was significantly induced by lipid overload in a time-dependent manner. However, upon co-administration of NaHS with the lipid mixture, the increase in the number of SA-β-gal-positive cells was remarkably reduced (**Fig. 15A**). Furthermore, the levels of other senescence-related proteins (p53 and p21) were also up-regulated in H9C2 cells in response to sustained lipid mix administration, which could be reversed by incubation with exogenously applied NaHS (**Fig. 15B**). These findings suggest that H_2S could attenuate lipid overload-induced cell senescence.

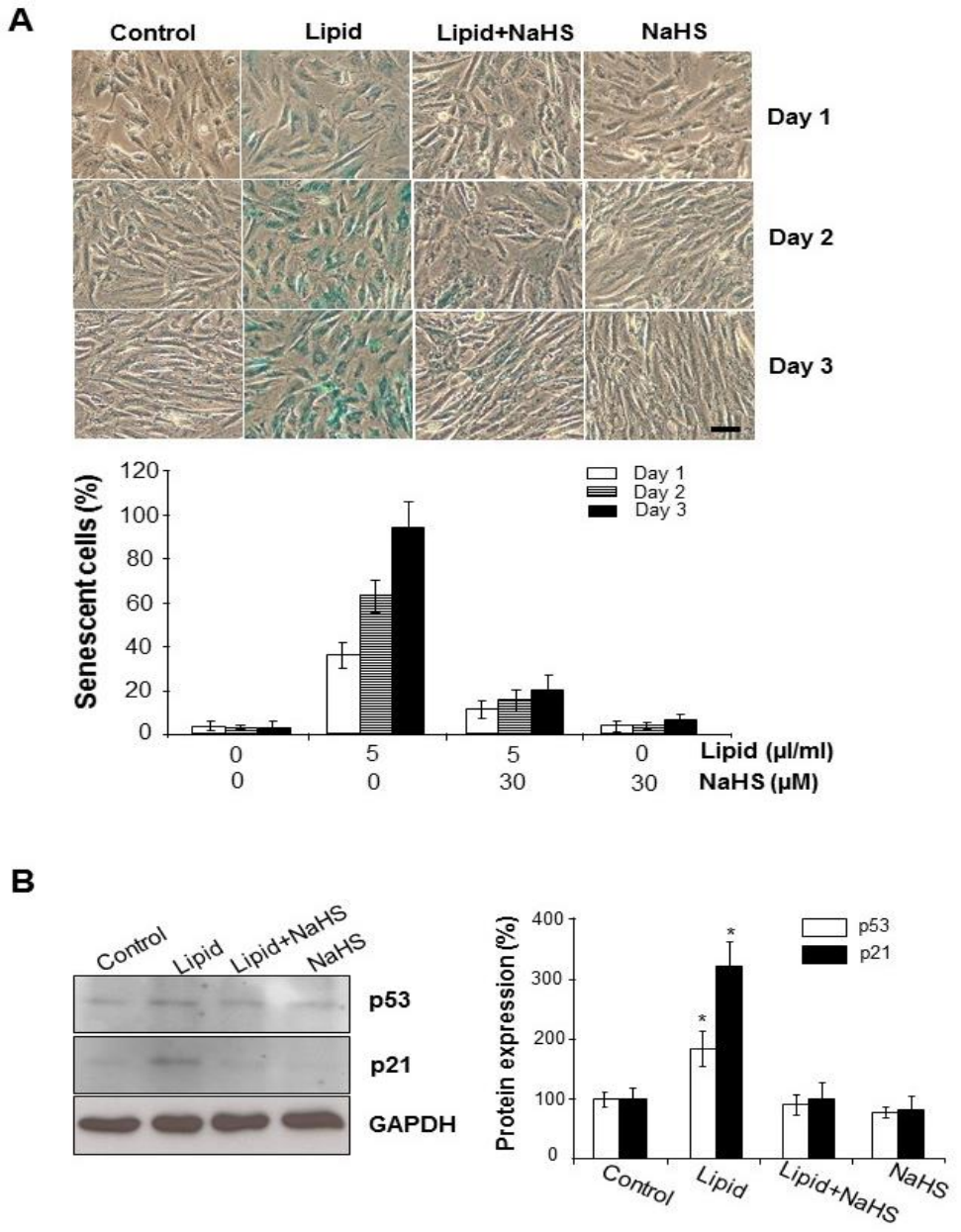

Fig. 15. H_2S protects H9C2 cells from lipid overload-induced senescence. After H9C2 cells were incubated with 5 μl/ml lipid mix without or with NaHS of 30 μM for three days, the cells were subjected to detection of cellular senescence (***A***) with a senescence β-galactosidase staining kit as well as (***B***) p21 and p53 protein expressions by western blotting. Scale bar: 20 μm. n=4. * $p<0.05$ versus all other groups.

4.5. H_2S inhibits lipid overload-upregulated acetyl-CoA and protein acetylation

Acetyl-CoA is a significant carrier for lipid metabolism and a critical regulator of protein acetylation in cells (Cai and Tu, 2011; Shi and Tu, 2015). A coupled enzyme assay was conducted to determine the acetyl-CoA content in cardiac cells. Our analysis revealed that lipid-overload incubation evidently enhanced cellular acetyl-CoA content, whereas NaHS treatment decreased the content of acetyl-CoA, even in the presence of the lipid mixture. We also found a slightly reduced level of acetyl-CoA by NaHS treatment alone (without statistical significance) (**Fig. 16A**).

Following the acetyl-CoA content change, we assessed the global acetylated lysine level and histone acetylation in H9C2 cells with lipid overload. As reflected in **Fig. 16B**, the global acetylated lysine level was remarkably up-regulated in the cells treated with lipid overload. Conversely, lipid overload-induced increase of global protein acetylation was attenuated by NaHS co-treatment. Unexpectedly, histone H3 acetylation level was not altered by either lipid mix or NaHS treatment, although the content of acetyl-CoA and the part of HDACs tested later have changed by adding lipid mixture or NaHS.

We next examined the effect of lipid and NaHS treatment on mRNA expressions related to protein acetylation. As illustrated in **Fig. 16C**, the transcript levels of HDACs 1, 2, 3, which belong to the class I histone deacetylases, were remarkably up-regulated by the

treatment with a lipid mixture. At the same time, exogenous application of NaHS blocked the upregulation of HDAC transcripts by lipid overload. Moreover, we examined the role of H_2S on mRNA expression of SIRT1, a class III histone deacetylase. The data demonstrated that SIRT1 transcript levels were slightly increased by lipid overload and inhibited by NaHS co-incubation, although these changes were not statistically significant. Neither lipid overload nor NaHS changed the transcription of the KAT gene, a central enzyme catalyzing protein acetylation (**Fig. 16C**). In combination, these results indicate that H_2S inhibits lipid overload-upregulated global protein acetylation, possibly by reducing acetyl-CoA content and suppressing class I HDACs.

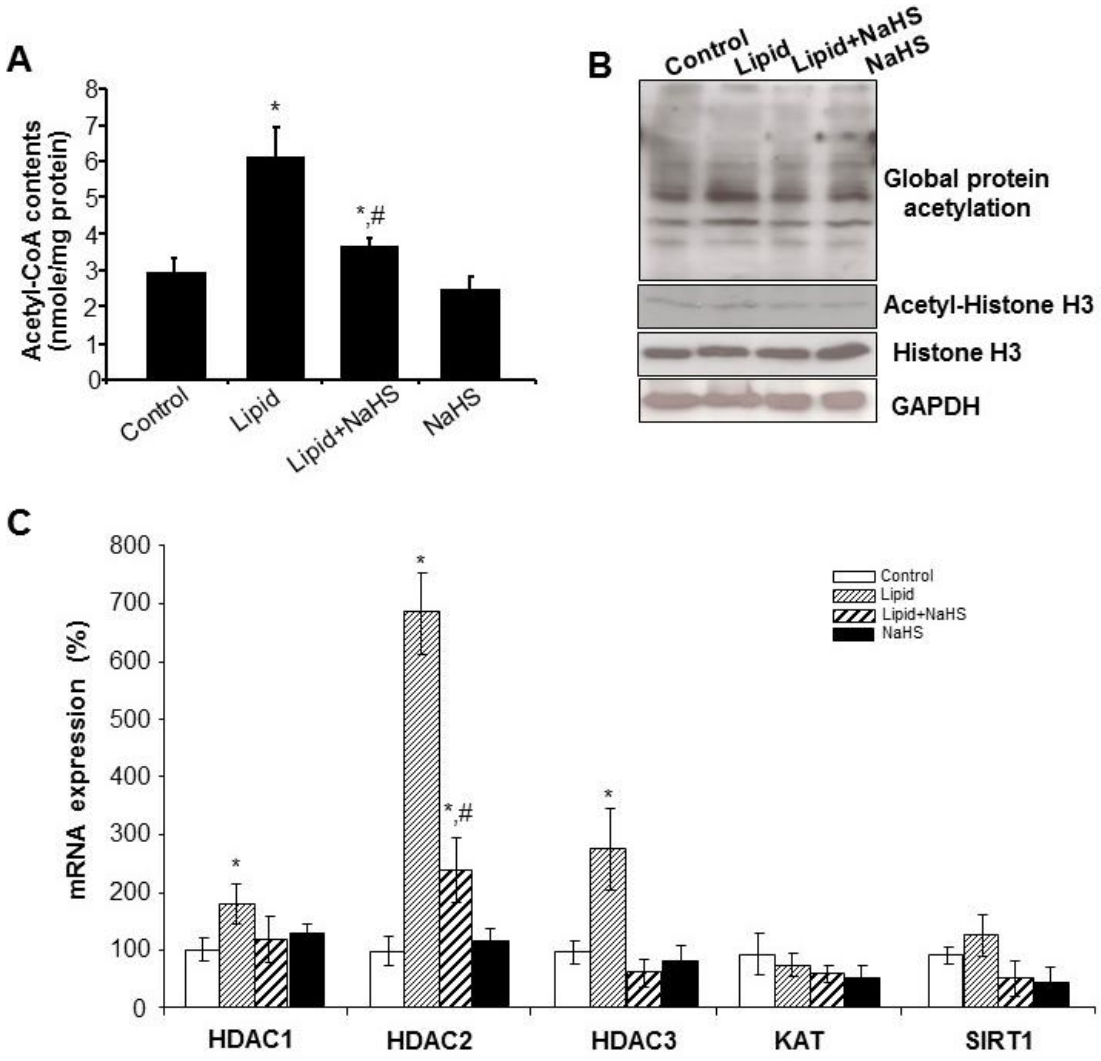

Fig. 16. H_2S inhibits lipid overload-upregulated acetyl-CoA and protein acetylation.

After incubating the cells with 5 μl/ml lipid mix without or with 30 μM NaHS for three days, the acetyl-CoA was detected using the coupled enzyme assay (***A***), and protein acetylation by western blotting (***B***), and the mRNA expressions of acetylation-related genes by real-time PCR (***C***). *, $p < 0.05$ vs. control cells; #, $p < 0.05$ vs. lipid group.

4.6. Acetate reverses the inhibitory role of H_2S on lipid overload-induced cellular senescence by enhancing protein acetylation

We next turned our attention to the mechanism of H_2S in cardiac cell senescence. H9C2 cells were incubated with lipid mix (5 μl/ml), NaHS (30 μM), and/or sodium acetate (1 mM) for 72 hours. As shown in **Fig. 17A**, the addition of sodium acetate reversed the inhibitory role of NaHS on lipid overload-induced cell senescence. As well, the protein level of senescence-related protein p21 was also increased in the H9C2 cells in response to sodium acetate administration in the presence of lipid mix and NaHS (**Fig. 17B**). Sodium acetate also significantly increased total cellular acetylated lysine. These data indicate that acetate reverses the inhibitory effect of H_2S on cellular senescence induced by lipid overload, likely by enhancing protein acetylation.

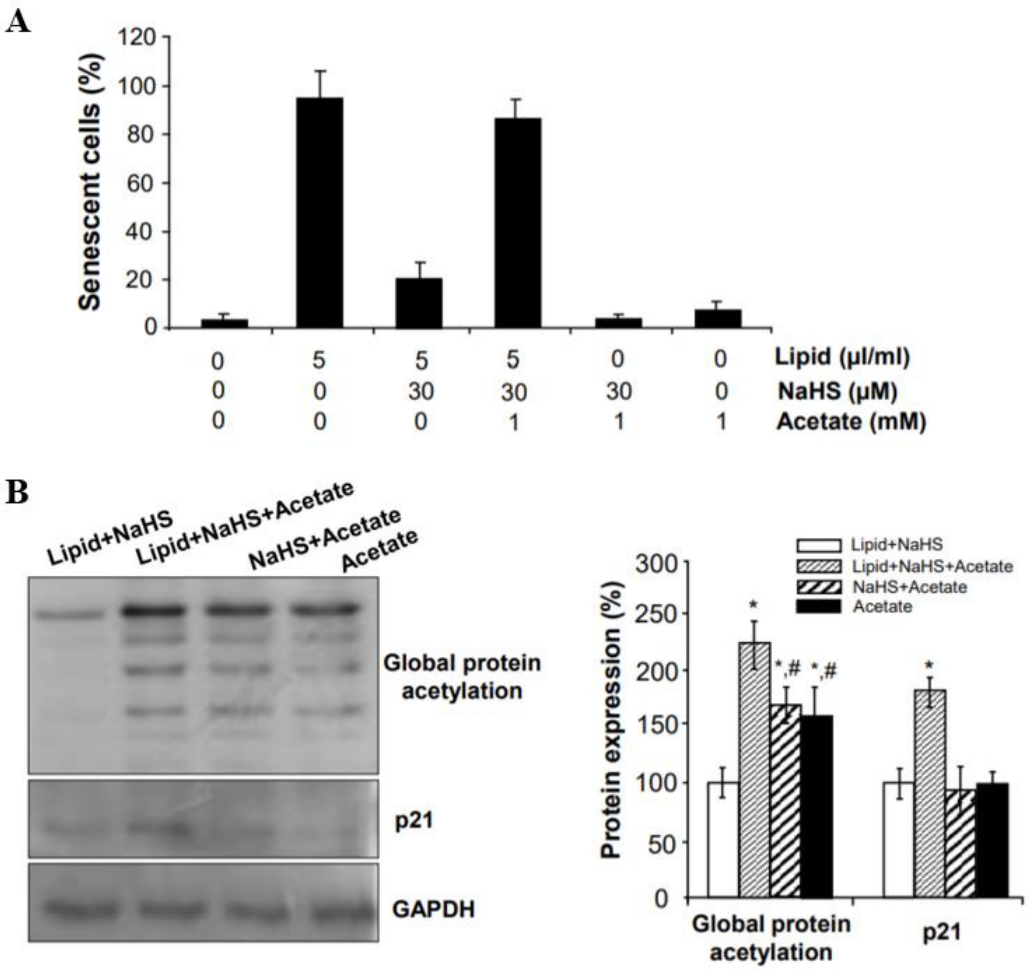

Fig. 17. Acetate reverses the inhibitory role of H_2S on lipid overload-induced cellular senescence by enhancing protein acetylation. After incubating the H9C2 cells with 30 μM NaHS, 5 μl/ml lipid mix, and/or 1 mM acetate sodium for three days, the cells were subjected to detection of cellular senescence (***A***) with a senescence β-galactosidase staining kit as well as protein acetylation and p21 expression (***B***) by western blotting. Scale bar: 20 μm. n=4.

4.7. CSE deficiency strengthens cardiac lipid accumulation, protein acetylation, and cellular ageing in HFD-fed mice

To test the influences of the CSE/H_2S system on lipid accumulation *in vivo*, CSE KO mice and WT littermates were fed with a high-fat diet or a control chow diet for 12 weeks. Direct measurement of triglyceride content revealed that high-fat feeding markedly increased the triglyceride content in both types of mice compared to the control group (**Fig. 18A**). Besides, in the control group, compared to WT mice, CSE KO mice had slightly higher triglyceride levels in their hearts. However, the lipid deposition in CSE-KO mice fed a high-fat diet was evidently more elevated than that in WT mice. It was further observed that the mice fed with a high-fat diet had higher expression of proteins related to lipid biogenesis in heart tissues, such as ACL and AceCS1. Mice possessing a CSE deficiency significantly up-regulated these enzymes when fed a high-fat diet (**Fig. 18B**). However, the expression of myocardial ACSL1 protein was not affected by either a high-fat diet or CSE deficiency. We further noticed that high-fat diet-fed mice had increased contents of cardiac acetyl-CoA in both types of mice compared to those with a control diet (**Fig. 18C**). The CSE knockout rose the stimulatory effect of a high-fat diet on the content of acetyl-CoA, consistent with the *in vitro* results. Then, the impact of CSE deficiency and a high-fat diet on protein acetylation levels was assessed in the heart tissue. The results indicated that feeding mice a high-fat diet for 12 weeks up-regulated the global protein acetylation level of WT hearts and led to a more significant increase in CSE-KO hearts (**Fig. 18D**). We further found that CSE deficiency had no effect on the levels of histone acetylation in heart tissues in both the high-fat diet group and control diet group, although a high-fat diet induced more histone acetylation at the 18^{th} lysine residue (**Fig. 18D**). The protein levels of senescence-related proteins p53 and p21 were

then detected. As shown in **Fig. 18E**, high-fat diet feeding up-regulated the p21 and p53 protein expression level, and the CSE knockout increased the stimulatory role of high-fat diet on p21 and p53 expression level in heart tissues. To sum up, these data indicate that the CSE deficiency promoted cardiac lipid accumulation, protein acetylation, and cellular ageing in mice fed a high-fat diet. This strongly suggests that the CSE/H_2S system is critical for maintaining lipid homeostasis and cellular senescence in heart tissues under lipid overload.

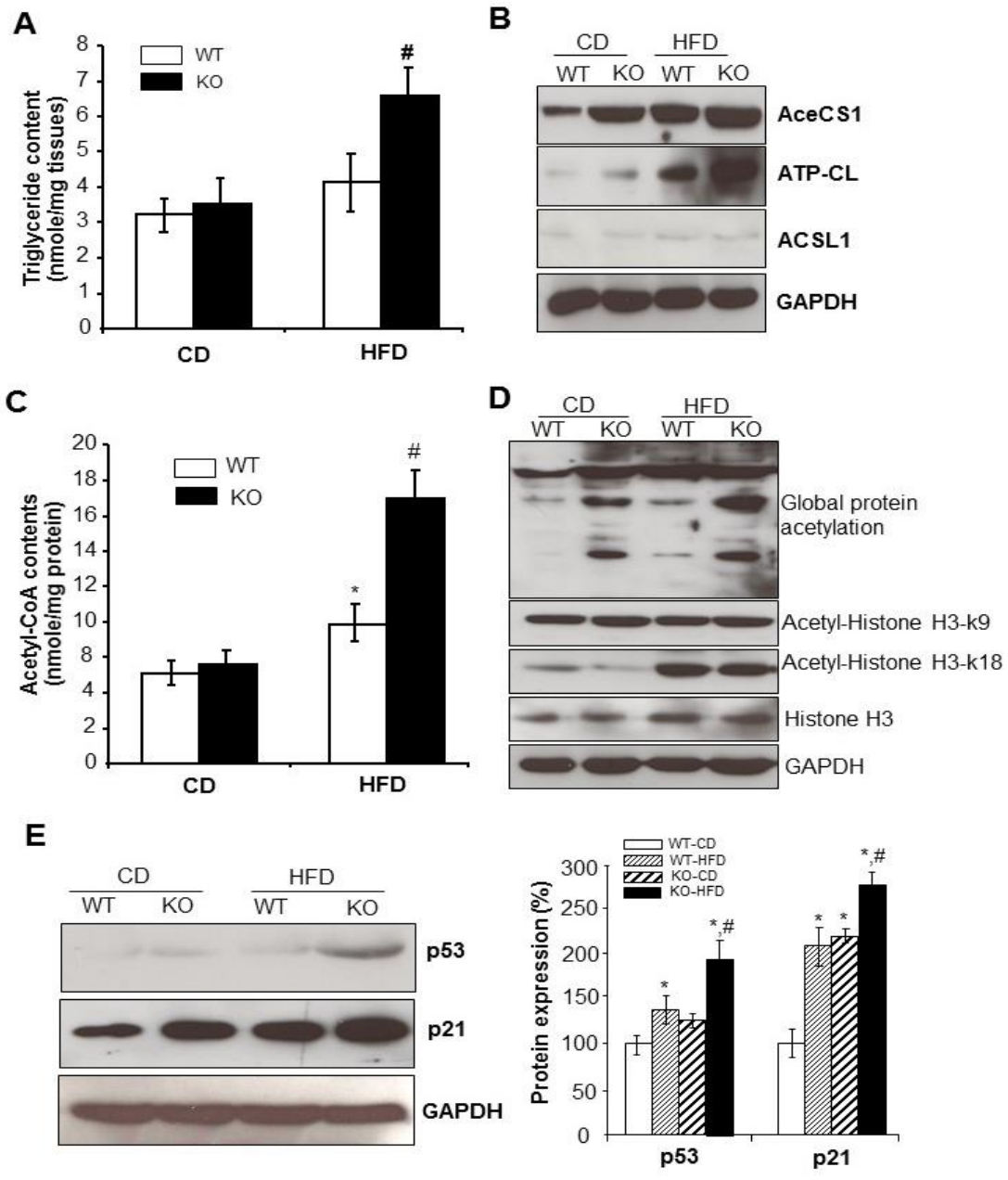

Fig. 18. CSE deficiency strengthens cardiac lipid accumulation, protein acetylation, and cellular ageing in HFD-fed mice. After feeding the mice with a high-fat diet (HFD) or control diet (CD) for 16 weeks, the heart tissues were collected for analysis of cellular triglyceride (***A***), expressions of lipid-metabolism related genes (***B***), acetyl-CoA contents (***C***) protein acetylation (***D***), and p21 and p53 protein expressions (***E***). At least 4 mice were utilized in each group. *, $p < 0.05$ vs. WT-CD; #, $p < 0.05$ vs. WT-HFD.

5. Discussion

Heart disorders are often observed in older people, and obesity is a driving factor for increasing the risk of cardiac remodelling and dysfunctions in the ageing heart (Hemanthakumar et al., 2021; Wakabayashi et al., 2019). The present study shows that H_2S, a novel gasotransmitter, can protect from lipid overload-induced cardiolipotoxicity and cellular senescence.

5.1. H_2S regulation of lipid accumulation

Under normal physiological conditions, cardiomyocytes meet approximately 60–80 percent of their energy demands via mitochondrial β-oxidation of the fatty acids. The metabolism of lipids is a complex process, which is composed of lipid uptake, generation, transport as well as oxidation. Lipids play critical roles in cardiac structure and function. Previous studies have revealed that an increase in circulating FFAs and TAGs is one of the

earliest consequences of obesity (Lopaschuk et al., 2007; Lopaschuk et al., 2010). High circulating fatty acids and TAGs can induce activation of the cardiac transcription factor PPARα as fatty acids are endogenous PPARα ligands, leading to enhanced uptake of myocardial fatty acid and their β- oxidation (Buchanan et al., 2005; Lopaschuk et al., 1994; Luiken et al., 2001; Young et al., 2002). Nevertheless, so far, the impact of H_2S on heart lipid metabolism was unclear.

This study provided evidence that H9C2 cell exposure to lipid mixtures caused an increase in intracellular lipids. The administration of NaHS at a physiologically relevant concentration (30 μM) prevented this elevation in lipid accumulation. A high-fat diet induced a higher triglyceride content in heart tissues from CSE KO mice than WT mice. Compared to untreated control mice, a slight increase in cardiac triglycerides level was found in untreated CSE KO mice. These results suggest that a CSE deficiency augments lipid accumulation in heart tissues, which can then be exacerbated in these mice under the condition of lipid overload. Consistent with this view, a recent research study revealed an evident increase in blood cholesterol and total liver lipids in CSE KO mice fed a high-fat diet compared to WT mice (Mani et al., 2015).

To investigate the mechanism by which H_2S affects lipid accumulation, we studied changes in the expression of genes related to lipid metabolisms, such as lipogenesis, lipolysis

and lipid transport. Firstly, we found that the treatment with a lipid mixture remarkably increased the expression of AceCS1 and ATP-Cl at the protein level in H9C2 cells. In contrast, the stimulatory effect of the lipid mix on the level of these protein expressions was blocked by exogenous NaHS treatment. Simultaneously, a high-fat diet can increase the expression of ATP-Cl and AceCS1 in the hearts of mice lacking CSE, suggesting that H_2S can reduce de novo fatty acid synthesis. Secondly, we confirmed that the presence of lipid overload increased mRNA expression of PPARα and ACOX1, which was attenuated by co-incubation with NaHS. These data suggest that H_2S lowers lipid overload-induced fatty acid β-oxidation. Thirdly, H_2S reversed the inhibitory role of lipid overload-induced ApoB mRNA expression. It can be inferred that H_2S would maintain intracellular triglyceride levels by stimulating lipid outflux. Consistent with our finding, it has been previously reported that in the HepG2 cells, H_2S partially inhibited the accumulation of hepatic lipid via promoting ApoB-mediated secretion (Ali et al., 2020). In combination, heart tissue can evolve strategies to prevent the adverse effects of excessive lipids through maintaining the balance of fatty acid biosynthesis, β-oxidation and transport, and a proper level of CSE/H_2S signalling is essential for this process.

5.2. H_2S regulation of protein acetylation

Lysine acetylation is a reversible and dynamic post-translational modification that affects different cellular processes such as energy metabolism, nuclear transcription, cell survival, and apoptosis (Hebert et al., 2013; Hirschey et al., 2010; Zhang et al., 2009). Such

modification appears when transferring an acetyl group to a target lysine residue via either enzymatic acetylation with acetyltransferase or non-enzymatic chemical modification of acetyl-CoA, thereby neutralizing the positive charge of lysine and altering protein interactions and structure (Shaw et al., 2008; Xiong and Guan, 2012). Acetyl-CoA is a kind of carrier for lipid metabolism and an essential regulator of protein acetylation in cells (Cai and Tu, 2011; Shi and Tu, 2015). It has been reported that the CSE/H_2S system can suppress hepatic acetyl-CoA accumulation caused by a high-fat diet (Ali et al., 2020). Here, we also confirmed that the CSE/H_2S system could reduce total protein acetylation by decreasing total intracellular acetyl-CoA content induced by lipid overload, further protecting H9C2 cells from cellular senescence. To further verify the relationship between H_2S and protein acetylation, we then used qRT-PCR to quantify the mRNA expression of acetylation-related genes in response to lipid overload. We observed that the transcripts of HDAC 1, 2, 3, which belong to the class I histone deacetylases, were increased in the presence of lipid mix but reduced by co-incubation with the H_2S donor, NaHS. SIRTs are class III histone deacetylases that catalyze the deacetylation reaction with NAD^+ as the cofactor (Scher et al., 2007). The current work observed that SIRT1 was not affected by either lipid overload or NaHS treatment. In contrast, another study reported that endogenous H_2S (CSE overexpression) and exogenous H_2S (H_2S releasing compounds GYY4137 and NaHS) increase the expression of SIRT1 and its deacetylase activity in the HepG2 cells (Du et al., 2019). Such differences probably are associated with the type of cells or H_2S donor used since the physiological concentration of H_2S and sensitivity to H_2S varies for different kinds of cells (Panthi et al., 2016). Besides, the

H_2S release rate within several donors is different (Powell et al., 2018). In summary, by changing the acetyl-CoA content in cells and the overall protein acetylation level, H_2S appears essential for inhibiting cellular senescence. The specific proteins modified via acetylation would need to be further investigated.

5.3. H_2S regulation of ROS and mitochondrial functions

Under physiological conditions, endogenous antioxidant systems can effectively decrease the level of ROS produced by cell oxidation, thus avoiding the adverse effects of high concentrations of ROS on cells (Kabel, 2014; Li et al., 2012; Moldogazieva et al., 2019; Niki, 2016; Valko et al., 2007). Here, we observed that excessive lipid accumulation promoted ROS production in hearts, and H_2S at a physiologically relevant concentration significantly reversed lipid overload-induced intracellular ROS production and lipid peroxidation. Consistent with this discovery, a previous study also revealed that H_2S possesses a protective effect against ferroptosis caused by RSL3 in the C2C12 cells, partially via inhibiting lipid peroxidation mediated by ROS (Wang et al., 2021). Xie. et al. confirmed that H_2S inhibited the production of mitochondrial ROS through p66Shc-dependent signal transduction (Xie et al., 2014b). Taken together, these results suggest that H_2S can decrease the harmful effects caused by oxidative stress. Nevertheless, the effects of H_2S on redox states are very different. H_2S has been reported to be a strong prooxidant. For instance, exposure to 0.5 mM NaHS leads to rapid cell necrosis and hepatocyte toxicity via inhibiting cytochrome oxidase followed by ROS production (Truong et al., 2006). It was found that H_2S at a dose of

more than 200 μM promotes DNA damage, probably by producing ROS at high levels in several cell lines, including glioblastoma cells along with non-transformed intestinal epithelial cells (Attene-Ramos et al., 2010; Jiang et al., 2016; Lazarević et al., 2018; Xiao et al., 2019).

Mitochondria are well established as the organelles responsible for oxidative phosphorylation and ATP production, which meets approximately 80 percent of cell energy needs (Papa, 1996). It has been shown that in obesity, excessive lipid deposition enhanced ROS production and lipid peroxidation, which subsequently caused damage to mitochondrial function and structure (Su et al., 2019). Accumulated reports indicate that H_2S biosynthetic enzymes exist in mitochondria. 3-MST exists in mitochondria and cytoplasm, while CBS and CSE mainly present in the cytoplasm, but they can also be translocated into mitochondria under stress conditions (Fu et al., 2012). Low concentrations of H_2S affect mitochondrial respiration, biogenesis, and morphology (Módis et al., 2016). Here we demonstrated that lipid overload reduced mitochondrial membrane potential, and that was reversed by supplementing H_2S. The reduction of mitochondrial membrane potential has been shown to promote mitochondrial structure abnormalities to some extent (Wang et al., 2017). In the current studies, we showed that lipid overload increased the expression of PPARα and ACOX1, which may promote fatty acid oxidation in mitochondria. This excessive lipid oxidation then leads to more ROS and reduced mitochondrial function. Reversal of lipid oxidation by NaHS administration reduced the damage of lipid overload to mitochondrial function and structure. Consistent with our findings, a previous study showed that the mitochondrion-targeted H_2S

donors AP39 and AP123 successfully decreased mitochondrial membrane hyperpolarization and ROS production, increased ETC complex III activity and mitochondrial metabolism in the endothelial cells (Gerő et al., 2016). Teng et al. also found that ischemia/hypoxia significantly increased mitochondrial CBS levels in rat liver, leading to elevated mitochondrial H_2S generation and ATP production (Teng et al., 2013). In cardiac cells, H_2S protected from H_2O_2-induced oxidative stress and restored mitochondrial membrane potential ψm (Zhang et al., 2019). However, at high levels, H_2S can inhibit the ETC, perturb redox balance, shift metabolism toward reductive carboxylation, and inhibit cellular proliferation (Libiad et al., 2019). Similarly, it has been demonstrated that H_2S disturbs energy and redox homeostasis in rat cerebral cortex mitochondria at toxic levels.

In conclusion, the findings mentioned above provide a strong indication that the physiologic level of H_2S appears to be critical for normal cellular metabolism and redox homeostasis by regulating mitochondrial function and ROS generation. A more thorough understanding of H_2S signalling in mitochondria is crucial for developing therapeutics against a whole host of disease states. Therefore, exogenous H_2S treatment or specific H_2S therapeutic targeting may prove valuable in the clinical setting to treat myocardial injury associated with ROS stress and mitochondrial function. The effect of H_2S and enzymes generating H_2S in the regulation of the cellular microenvironment and how they relate to both normal and diseased states would be an exciting area for future exploration.

5.4. CSE deficiency, cardiac senescence, and heart disorders

The cardioprotective roles of the CSE/H_2S system have been well explored (Patel et al., 2009; Yang et al., 2005). The current study offers evidence that the CSE/H_2S system is significant for improving cardiac ageing under the condition of lipid overload. SA-β-gal and p53/p21 expressions are commonly used markers to detect cell senescence (Hernandez-Segura et al., 2018). In addition, there are markers such as morphological changes, chromatin alteration, gene expression changes, senescence-associated secretory phenotype (SASP), cell cycle arrest, DNA damage and DNA damage responses (Lee et al., 2006). It should be mentioned that these senescence biomarkers are not unique to senescent cells because some markers also can be seen in quiescent or apoptotic cells (Rufini et al., 2013; Zhang et al., 2018). For example, SA-β-gal, the most widely accepted biomarker for cell senescence, is based on β-galactosidase activity detected at pH 6.0 (Lee et al., 2006). However, some factors that lead to increased lysosome content may also cause cells to exhibit increased β-galactosidase activity at pH 6.0, even if the cells are not senescent (Severino et al., 2000; Untergasser et al., 2003). Therefore, the recognition of cellular senescence should be based on the detection of several biomarkers. In the present study, we found a high level of SA-β-gal activity ROS generation, upregulated p53 and p21 expression, increased cell size and flattening when cells were exposed to high lipid mixtures. Besides, after 12 weeks of high-fat diet feeding, the heart tissues of CSE-KO mice showed significantly increased lipid deposition and expressed cell senescence markers. To confirm that cardiomyocytes do

undergo senescence when exposed to large amounts of lipid mixtures, further testing of other markers, such as DNA damage and SASP, is needed.

We did not assess heart function alteration in CSE-KO mice under high-fat diet feeding in this present work. Previous studies have reported that high levels of myocardial triglycerides were related to adverse cardiac outcomes, including reduced diastolic function with preserved ejection fraction (Kar et al., 2019). Deletion of CSE has been shown to exacerbate myocardial ischemia injury after ischemia-reperfusion (Elrod et al., 2007). The ageing CSE-KO mice revealed more severe oxidative stress and left ventricular remodelling than WT mice. Conversely, cardiac-specific CSE expression can protect heart function and structure after transverse aortic contraction by increasing mitochondrial homeostasis, NO/cGMP signalling, and decreasing oxidative stress (Kondo et al., 2013). Another study suggested that the pathophysiological process of myocardial ischemia injury induced by ligation is related to endogenous CSE or H_2S impairment (Xie et al., 2014a). Exogenous H_2S can effectively protect myocardial cells and contractile activity and limit the myocardial infarction scope. The protective effect of H_2S on myocardial ischemia injury was attributed to its mitochondrial function protection and antioxidant activities (Xie et al., 2014a). The level of cardiac H_2S is lower in ageing diabetic mice and human samples of hypertrophic myocardium. All these findings point to the beneficial role of H_2S against cardiac ageing and dysfunctions. The link between defects in H_2S signalling pathways and cardiac lipotoxicity

and the role of H_2S in attenuating lipid overload-induced cardiac ageing require further investigation.

6. Conclusion

In summary, obesity is a leading risk factor for heart dysfunction, and one of the mechanisms is the interruption of lipid metabolism. This work explored the regulatory effects of the CSE/H_2S system on lipid overload-induced lipotoxicity and cardiac senescence. The most significant finding of this study is that incubation of H9C2 (rat cardiomyocyte cells) with a lipid mixture inhibited cell viability and promoted the cellular accumulation of lipids, generation of ROS, mitochondrial dysfunctions, and lipid peroxidation, all of which could be reversed by co-incubation with exogenously applied NaHS (an H_2S donor). Further data revealed that H_2S protected H9C2 cells from lipid overload-induced senescence by altering the expressions of genes related to lipid metabolism and inhibiting the acetyl-CoA production and the overall protein acetylation level (**Fig. 19**). *In vivo*, knockout of the CSE gene strengthened cardiac lipid accumulation, protein acetylation, and cellular ageing in mice fed a high-fat diet. Taken together, the CSE/H_2S system is essential for maintaining lipid homeostasis in cardiomyocytes and in reducing cellular senescence in heart cells under lipid overload.

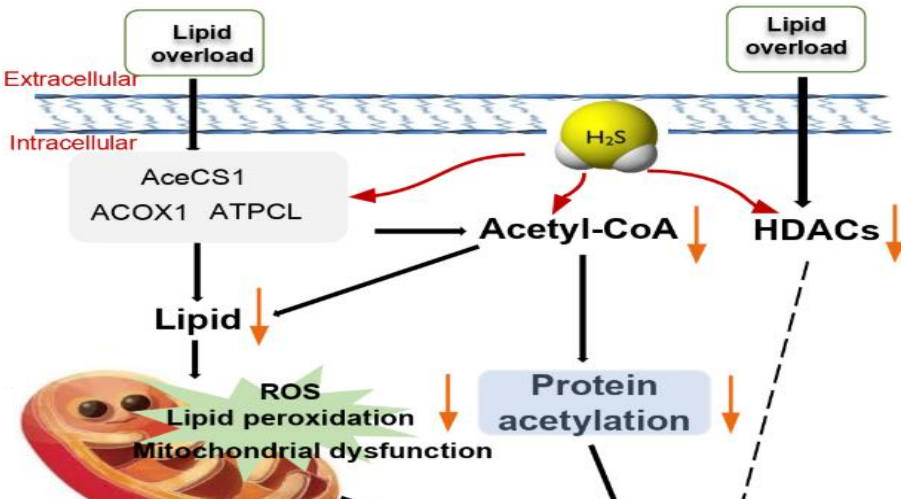

Fig. 19. The proposed protective mechanism of CSE/H_2S signal on lipotoxicity and cardiac cell senescence.

7. Limitation of this study and future work

H_2S is an endogenous gas messenger that can regulate a variety of physiological processes. However, the influences of H_2S against heart health have only recently been discovered. While the underlying mechanisms are not fully explored, this research provides insight into how CSE/ H_2S may regulate cardiac lipotoxicity and senescence.

One limitation of this study is that we used a fast-releasing H_2S donor NaHS. One-third of H_2S is released from NaHS within seconds when added to the medium. This suggests that the real H_2S inside the medium cannot keep a stable concentration for a long time. To overcome this limitation, a slow and steady H_2S-producing donor, such as GYY4137 could be investigated as a potential alternative to NaHS.

MTT is a yellow chemical that produces purple formazan crystals under respiratory-related enzymes in the mitochondria of living cells (Angius and Floris, 2015; Kumar et al., 2018). Under normal circumstances, since the number of formazan crystals produced represents the dynamics of mitochondria and is directly proportional to the number of living cells, the absorbance can be used to evaluate how many cells there are (Kumar et al., 2018; Stockert et al., 2012). When the cell reached 50% confluence in the present study, we treated cells with lipid mix and NaHS and detected cell viability after treatment 1-3 days. On the 2nd and 3rd days after the treatment, we found that the cell viability in the control group increased faster than the lipid group, which means lipid overload suppressed the increase in the cell viability. We also found that the concentration of lipid mixture we used did not induce cell apoptosis. In subsequent senescence detection, it was found that lipids overload-induced cardiac cell senescence and H_2S can protect cells from lipid overload-induced senescence. This explained why cell viability in the lipid group increased slower than the control group when lipids did not cause cell apoptosis. But the problem is that the MTT assay evaluates the number of living cells based on the activity of mitochondria (Kumar et al., 2018). In our current investigation, lipid overload induced mitochondrial dysfunction. In this case, the decrease in absorbance in the MTT assay is due to lipid-induced cell senescence lowering cell reproduction and damage to mitochondrial function. In the future, BrdU cell proliferation assay may be used for cell number measurement, which is based on the detection of newly synthesized DNA (Diermeier-Daucher et al., 2009). It can accurately reflect the reproduction of cells.

Future studies need to identify the specific proteins acetylated and how H_2S may affect protein acetylation. A large number of cellular proteins can be acetylated and may impact cell viability, cell senescence, ROS generation, and cellular lipid metabolism. The altered lipid intermediates by H_2S also need to be explored. This study demonstrated that H_2S could protect H9C2 cells from senescence induced by lipid overload, possibly through its ability to inhibit protein acetylation. We also found that histone deacetylases are regulated by lipid/NaSH. Whether H_2S could protect H9C2 cells from lipid overload-induced senescence by regulating specific histone deacetylases needs to be further explored. Further clinical studies on the effects of H_2S signalling pathways on lipid metabolism and heart disease need to be conducted.

8. References

Abel, E.D., Litwin, S.E., and Sweeney, G. (2008) "Cardiac remodeling in obesity." *Physiol Rev.* **88**, 389-419.

Abe, K., and Kimura, H. (1996) "The possible role of hydrogen sulfide as an endogenous neuromodulator." *J Neurosci.* **16**, 1066-1071.

Abdurrachim, D., Ciapaite, J., Wessels, B., Nabben, M., Luiken, J.J., Nicolay, K., and Prompers, J.J. (2014) "Cardiac diastolic dysfunction in high-fat diet fed mice is

associated with lipotoxicity without impairment of cardiac energetics in vivo." *Biochim Biophys Acta.* **1842**, 1525-1537.

Ago, T., Kuroda, J., Pain, J., Fu, C., Li, H., and Sadoshima, J. (2010) "Upregulation of Nox4 by hypertrophic stimuli promotes apoptosis and mitochondrial dysfunction in cardiac myocytes." *Circ Res.* **106**, 1253-1264.

Ahmad, A., Gerö, D., Olah, G., and Szabo, C. (2016) "Effect of endotoxemia in mice genetically deficient in cystathionine-γ-lyase, cystathionine-β-synthase or 3-mercaptopyruvate sulfurtransferase." *Int J Mol Med.* **38**, 1683-1692.

Albertini, E., Kozieł, R., Dürr, A., Neuhaus, M., and Jansen-Dürr, P. (2012) "Cystathionine beta synthase modulates senescence of human endothelial cells." *Aging (Albany NY).* **4**, 664-673.

Albuquerque, D., Stice, E., Rodríguez-López, R., Manco, L., and Nóbrega, C. (2015) "Current review of genetics of human obesity: from molecular mechanisms to an evolutionary perspective." *Mol Genet Genomics.* **290**, 1191-1221.

Alcendor, R.R., Gao, S., Zhai, P., Zablocki, D., Holle, E., Yu, X., Tian, B., Wagner, T., Vatner, S.F., and Sadoshima, J. (2007) "Sirt1 regulates aging and resistance to oxidative stress in the heart." *Circ Res.* **100**, 1512-1521.

Ali, A., Zhang, Y., Fu, M., Pei, Y., Wu, L., Wang, R., and Yang, G. (2020) "Cystathionine gamma-lyase/H(2)S system suppresses hepatic acetyl-CoA accumulation and nonalcoholic fatty liver disease in mice." *Life Sci.* **252**, 117661.

Allsop, J., and Watts, R.W. (1975) "Methionine adenosyltransferase, cystathionine beta-synthase and cystathionine gamma-lyase activity of rat liver subcellular particles, human blood cells and mixed white cells from rat bone marrow." *Clin Sci Mol Med Suppl.* **48**, 509-513.

Alpert, M.A. (2001) "Obesity cardiomyopathy: pathophysiology and evolution of the clinical syndrome." *Am J Med Sci.* **321**, 225-236.

Alpert, M.A., Karthikeyan, K., Abdullah, O., and Ghadban, R. (2018) "Obesity and Cardiac Remodeling in Adults: Mechanisms and Clinical Implications." *Prog Cardiovasc Dis.* **61**, 114-123.

Alpert, M.A., Omran, J., Mehra, A., and Ardhanari, S. (2014) "Impact of obesity and weight loss on cardiac performance and morphology in adults." *Prog Cardiovasc Dis.* **56**, 391-400.

Andreyev, A.Y., Tsui, H.S., Milne, G.L., Shmanai, V.V., Bekish, A.V., Fomich, M.A., Pham, M.N., Nong, Y., Murphy, A.N., Clarke, C.F., et al. (2015) "Isotope-reinforced polyunsaturated fatty acids protect mitochondria from oxidative stress." *Free Radic Biol Med.* **82**, 63-72.

Angius, F., and Floris, A. (2015) "Liposomes and MTT cell viability assay: an incompatible affair." *Toxicol In Vitro.* **29**, 314-319.

Aon, M.A., Cortassa, S., Akar, F.G., and O'Rourke, B. (2006) "Mitochondrial criticality: a new concept at the turning point of life or death." *Biochim Biophys Acta.* **1762**, 232-240.

Attene-Ramos, M.S., Nava, G.M., Muellner, M.G., Wagner, E.D., Plewa, M.J., and Gaskins, H.R. (2010) "DNA damage and toxicogenomic analyses of hydrogen sulfide in human intestinal epithelial FHs 74 Int cells." *Environ Mol Mutagen.* **51**, 304-314.

Aune, S.E., Herr, D.J., Mani, S.K., and Menick, D.R. (2014) "Selective inhibition of class I but not class IIb histone deacetylases exerts cardiac protection from ischemia reperfusion." *J Mol Cell Cardiol.* **72**, 138-145.

Aurich, A.C., Niemann, B., Pan, R., Gruenler, S., Issa, H., Silber, R.E., and Rohrbach, S. (2013) "Age-dependent effects of high fat-diet on murine left ventricles: role of palmitate." *Basic Res Cardiol.* **108**, 369.

Bannister, A.J., and Kouzarides, T. (2011) "Regulation of chromatin by histone modifications." *Cell Res.* **21**, 381-395.

Baskar, R., Sparatore, A., Del Soldato, P., and Moore, P.K. (2008) "Effect of S-diclofenac, a novel hydrogen sulfide releasing derivative inhibit rat vascular smooth muscle cell proliferation." *Eur J Pharmacol.* **594**, 1-8.

Battiprolu, P.K., Hojayev, B., Jiang, N., Wang, Z.V., Luo, X., Iglewski, M., Shelton, J.M., Gerard, R.D., Rothermel, B.A., Gillette, T.G., et al. (2012) "Metabolic stress-induced

activation of FoxO1 triggers diabetic cardiomyopathy in mice." *J Clin Invest.* **122**, 1109-1118.

Benavides, G.A., Squadrito, G.L., Mills, R.W., Patel, H.D., Isbell, T.S., Patel, R.P., Darley-Usmar, V.M., Doeller, J.E., and Kraus, D.W. (2007) "Hydrogen sulfide mediates the vasoactivity of garlic." *Proc Natl Acad Sci U S A.* **104**, 17977-17982.

Bibli, S.I., Andreadou, I., Chatzianastasiou, A., Tzimas, C., Sanoudou, D., Kranias, E., Brouckaert, P., Coletta, C., Szabo, C., Kremastinos, D.T., et al. (2015) "Cardioprotection by H2S engages a cGMP-dependent protein kinase G/phospholamban pathway." *Cardiovasc Res.* **106**, 432-442.

Bjorkegren, J., Véniant, M., Kim, S.K., Withycombe, S.K., Wood, P.A., Hellerstein, M.K., Neese, R.A., and Young, S.G. (2001) "Lipoprotein secretion and triglyceride stores in the heart." *J Biol Chem.* **276**, 38511-38517.

Boengler, K., Kosiol, M., Mayr, M., Schulz, R., and Rohrbach, S. (2017) "Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue." *J Cachexia Sarcopenia Muscle.* **8**, 349-369.

Borlaug, B.A., Nishimura, R.A., Sorajja, P., Lam, C.S., and Redfield, M.M. (2010) "Exercise hemodynamics enhance diagnosis of early heart failure with preserved ejection fraction." *Circ Heart Fail.* **3**, 588-595.

Briones, A.M., Nguyen Dinh Cat, A., Callera, G.E., Yogi, A., Burger, D., He, Y., Corrêa, J.W., Gagnon, A.M., Gomez-Sanchez, C.E., Gomez-Sanchez, E.P., et al. (2012)

"Adipocytes produce aldosterone through calcineurin-dependent signaling pathways: implications in diabetes mellitus-associated obesity and vascular dysfunction." *Hypertension.* **59**, 1069-1078.

Buchanan, J., Mazumder, P.K., Hu, P., Chakrabarti, G., Roberts, M.W., Yun, U.J., Cooksey, R.C., Litwin, S.E., and Abel, E.D. (2005) "Reduced cardiac efficiency and altered substrate metabolism precedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity." *Endocrinology.* **146**, 5341-5349.

Burdon, R.H., Gill, V., and Alliangana, D. (1996) "Hydrogen peroxide in relation to proliferation and apoptosis in BHK-21 hamster fibroblasts." *Free Radic Res.* **24**, 81-93.

Cai, L., and Tu, B.P. (2011) "On acetyl-CoA as a gauge of cellular metabolic state." *Cold Spring Harb Symp Quant Biol.* **76**, 195-202.

Castañeda, D., Gabani, M., Choi, S.K., Nguyen, Q.M., Chen, C., Mapara, A., Kassan, A., Gonzalez, A.A., Ait-Aissa, K., and Kassan, M. (2019) "Targeting autophagy in obesity-associated heart disease." *Obesity (Silver Spring).* **27**, 1050-1058.

Cedar, H., and Bergman, Y. (2009) "Linking DNA methylation and histone modification: patterns and paradigms." *Nat Rev Genet.* **10**, 295-304.

Chatzianastasiou, A., Bibli, S.I., Andreadou, I., Efentakis, P., Kaludercic, N., Wood, M.E., Whiteman, M., Di Lisa, F., Daiber, A., Manolopoulos, V.G., et al. (2016)

"Cardioprotection by H2S Donors: Nitric Oxide-Dependent and‐Independent Mechanisms." *J Pharmacol Exp Ther.* **358**, 431-440.

Chen, X., Jhee, K.H., and Kruger, W.D. (2004) "Production of the neuromodulator H2S by cystathionine beta-synthase via the condensation of cysteine and homocysteine." *J Biol Chem.* **279**, 52082-52086.

Chen, X., Sun, K., Jiao, S., Cai, N., Zhao, X., Zou, H., Xie, Y., Wang, Z., Zhong, M., and Wei, L. (2014) "High levels of SIRT1 expression enhance tumorigenesis and associate with a poor prognosis of colorectal carcinoma patients." *Sci Rep.* **4**, 7481.

Chen, Y., Cai, G.H., Xia, B., Wang, X., Zhang, C.C., Xie, B.C., Shi, X.C., Liu, H., Lu, J.F., Zhang, R.X., et al. (2020) "Mitochondrial aconitase controls adipogenesis through mediation of cellular ATP production." *Faseb j.* **34**, 6688-6702.

Cheng, J., Nanayakkara, G., Shao, Y., Cueto, R., Wang, L., Yang, W.Y., Tian, Y., Wang, H., and Yang, X. (2017) "Mitochondrial Proton Leak Plays a Critical Role in Pathogenesis of Cardiovascular Diseases." *Adv Exp Med Biol.* **982**, 359-370.

Chiu, H.C., Kovacs, A., Ford, D.A., Hsu, F.F., Garcia, R., Herrero, P., Saffitz, J.E., and Schaffer, J.E. (2001) "A novel mouse model of lipotoxic cardiomyopathy." *J Clin Invest.* **107**, 813-822.

Christensen, D.G., Xie, X., Basisty, N., Byrnes, J., McSweeney, S., Schilling, B., and Wolfe, A.J. (2019) "Post-translational protein acetylation: An elegant mechanism for bacteria to dynamically regulate metabolic functions." *Frontiers in Microbiology.* **10**.

Christopher, M., Rantzau, C., Chen, Z.P., Snow, R., Kemp, B., and Alford, F.P. (2006) "Impact of in vivo fatty acid oxidation blockade on glucose turnover and muscle glucose metabolism during low-dose AICAR infusion." *Am J Physiol Endocrinol Metab.* **291**, E1131-1140.

Collado, M., Blasco, M.A., and Serrano, M. (2007) "Cellular senescence in cancer and aging." *Cell.* **130**, 223-233.

D'Souza, K., Nzirorera, C., and Kienesberger, P.C. (2016) "Lipid metabolism and signaling in cardiac lipotoxicity." *Biochim Biophys Acta.* **1861**, 1513-1524.

Dalmasso, G., Marin Zapata, P.A., Brady, N.R., and Hamacher-Brady, A. (2017) "Agent-Based Modeling of Mitochondria Links Sub-Cellular Dynamics to Cellular Homeostasis and Heterogeneity." *PLoS One.* **12**, e0168198.

DeFronzo, R.A. (2010) "Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009." *Diabetologia.* **53**, 1270-1287.

Diermeier-Daucher, S., Clarke, S.T., Hill, D., Vollmann-Zwerenz, A., Bradford, J.A., and Brockhoff, G. (2009) "Cell type specific applicability of 5-ethynyl-2'-deoxyuridine (EdU) for dynamic proliferation assessment in flow cytometry." *Cytometry A.* **75**, 535-546.

Donnarumma, E., Trivedi, R.K., and Lefer, D.J. (2017) "Protective Actions of H2S in Acute Myocardial Infarction and Heart Failure." *Compr Physiol.* **7**, 583-602.

Drosatos, K., and Schulze, P.C. (2013) "Cardiac lipotoxicity: molecular pathways and therapeutic implications." *Curr Heart Fail Rep.* **10**, 109-121.

Du, C., Lin, X., Xu, W., Zheng, F., Cai, J., Yang, J., Cui, Q., Tang, C., Cai, J., Xu, G., et al. (2019) "Sulfhydrated sirtuin-1 increasing its deacetylation activity is an essential epigenetics mechanism of anti-atherogenesis by hydrogen sulfide." *Antioxid Redox Signal.* **30**, 184-197.

Dugbartey, G.J. (2017) "Diabetic nephropathy: A potential savior with 'rotten-egg' smell." *Pharmacol Rep.* **69**, 331-339.

Ebong, I.A., Goff, D.C., Jr., Rodriguez, C.J., Chen, H., and Bertoni, A.G. (2014) "Mechanisms of heart failure in obesity." *Obes Res Clin Pract.* **8**, e540-548.

Eckel, R.H., Barouch, W.W., and Ershow, A.G. (2002) "Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on the pathophysiology of obesity-associated cardiovascular disease." *Circulation.* **105**, 2923-2928.

Elrod, J.W., Calvert, J.W., Morrison, J., Doeller, J.E., Kraus, D.W., Tao, L., Jiao, X., Scalia, R., Kiss, L., Szabo, C., et al. (2007) "Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function." *Proc Natl Acad Sci U S A.* **104**, 15560-15565.

Even, S.E., Dulak-Lis, M.G., Touyz, R.M., and Nguyen Dinh Cat, A. (2014) "Crosstalk between adipose tissue and blood vessels in cardiometabolic syndrome: implication of steroid hormone receptors (MR/GR)." *Horm Mol Biol Clin Investig.* **19**, 89-101.

Filipovic, M.R. (2015) "Persulfidation (S-sulfhydration) and H2S." *Handb Exp Pharmacol.* **230**, 29-59.

Filipovic, M.R., Zivanovic, J., Alvarez, B., and Banerjee, R. (2018) "Chemical Biology of H(2)S Signaling through Persulfidation." *Chem Rev.* **118**, 1253-1337.

Fiorucci, S., Distrutti, E., Cirino, G., and Wallace, J.L. (2006) "The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver." *Gastroenterology.* **131**, 259-271.

Francois, A., Canella, A., Marcho, L., and Stratton, M.S. (2021a) "Protein acetylation in cardiac aging." *J Mol Cell Cardiol.*

Francois, A., Canella, A., Marcho, L.M., and Stratton, M.S. (2021b) "Protein acetylation in cardiac aging." *J Mol Cell Cardiol.* **157**, 90-97.

Fu, M., Zhang, W., Wu, L., Yang, G., Li, H., and Wang, R. (2012) "Hydrogen sulfide (H2S) metabolism in mitochondria and its regulatory role in energy production." *Proc Natl Acad Sci U S A.* **109**, 2943-2948.

Furne, J., Saeed, A., and Levitt, M.D. (2008) "Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values." *Am J Physiol Regul Integr Comp Physiol.* **295**, R1479-1485.

Fushimi, T., Suruga, K., Oshima, Y., Fukiharu, M., Tsukamoto, Y., and Goda, T. (2006) "Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet." *Br J Nutr.* **95**, 916-924.

Gadalla, M.M., and Snyder, S.H. (2010) "Hydrogen sulfide as a gasotransmitter." *J Neurochem.* **113**, 14-26.

Gaggini, M., Saponaro, C., and Gastaldelli, A. (2015) "Not all fats are created equal: adipose vs. ectopic fat, implication in cardiometabolic diseases." *Horm Mol Biol Clin Investig.* **22**, 7-18.

Gallo, P., Latronico, M.V., Gallo, P., Grimaldi, S., Borgia, F., Todaro, M., Jones, P., Gallinari, P., De Francesco, R., Ciliberto, G., et al. (2008) "Inhibition of class I histone deacetylase with an apicidin derivative prevents cardiac hypertrophy and failure." *Cardiovasc Res.* **80**, 416-424.

Gerő, D., Torregrossa, R., Perry, A., Waters, A., Le-Trionnaire, S., Whatmore, J.L., Wood, M., and Whiteman, M. (2016) "The novel mitochondria-targeted hydrogen sulfide (H(2)S) donors AP123 and AP39 protect against hyperglycemic injury in microvascular endothelial cells in vitro." *Pharmacol Res.* **113**, 186-198.

Goh, K.Y., Qu, J., Hong, H., Liu, T., Dell'Italia, L.J., Wu, Y., O'Rourke, B., and Zhou, L. (2016) "Impaired mitochondrial network excitability in failing guinea-pig cardiomyocytes." *Cardiovasc Res.* **109**, 79-89.

Goldberg, I.J., Trent, C.M., and Schulze, P.C. (2012) "Lipid metabolism and toxicity in the heart." *Cell Metab.* **15**, 805-812.

Goubern, M., Andriamihaja, M., Nübel, T., Blachier, F., and Bouillaud, F. (2007) "Sulfide, the first inorganic substrate for human cells." *Faseb j.* **21**, 1699-1706.

Guo, W., Kan, J.T., Cheng, Z.Y., Chen, J.F., Shen, Y.Q., Xu, J., Wu, D., and Zhu, Y.Z. (2012) "Hydrogen sulfide as an endogenous modulator in mitochondria and mitochondria dysfunction." *Oxid Med Cell Longev.* **2012**, 878052.

Hankiewicz, J.H., Banke, N.H., Farjah, M., and Lewandowski, E.D. (2010) "Early impairment of transmural principal strains in the left ventricular wall after short-term, high-fat feeding of mice predisposed to cardiac steatosis." *Circ Cardiovasc Imaging.* **3**, 710-717.

Harayama, T., and Riezman, H. (2018) "Understanding the diversity of membrane lipid composition." *Nat Rev Mol Cell Biol.* **19**, 281-296.

Hassan, M.I., Boosen, M., Schaefer, L., Kozlowska, J., Eisel, F., von Knethen, A., Beck, M., Hemeida, R.A., El-Moselhy, M.A., Hamada, F.M., et al. (2012) "Platelet-derived growth factor-BB induces cystathionine γ-lyase expression in rat mesangial cells via a redox-dependent mechanism." *Br J Pharmacol.* **166**, 2231-2242.

Hauck, A.K., and Bernlohr, D.A. (2016) "Oxidative stress and lipotoxicity." *J Lipid Res.* **57**, 1976-1986.

Haykowsky, M.J., Kouba, E.J., Brubaker, P.H., Nicklas, B.J., Eggebeen, J., and Kitzman, D.W. (2014) "Skeletal muscle composition and its relation to exercise intolerance in older patients with heart failure and preserved ejection fraction." *Am J Cardiol.* **113**, 1211-1216.

Hebert, A.S., Dittenhafer-Reed, K.E., Yu, W., Bailey, D.J., Selen, E.S., Boersma, M.D., Carson, J.J., Tonelli, M., Balloon, A.J., Higbee, A.J., et al. (2013) "Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome." *Mol Cell.* **49**, 186-199.

Hemanthakumar, K.A., Fang, S., Anisimov, A., Mäyränpää, M.I., Mervaala, E., and Kivelä, R. (2021) "Cardiovascular disease risk factors induce mesenchymal features and senescence in mouse cardiac endothelial cells." *Elife.* **10**.

Hernandez-Segura, A., Nehme, J., and Demaria, M. (2018) "Hallmarks of cellular senescence." *Trends Cell Biol.* **28**, 436-453.

Hirschey, M.D., Shimazu, T., Goetzman, E., Jing, E., Schwer, B., Lombard, D.B., Grueter, C.A., Harris, C., Biddinger, S., Ilkayeva, O.R., et al. (2010) "SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation." *Nature.* **464**, 121-125.

Hopkins, M., and Blundell, J.E. (2016) "Energy balance, body composition, sedentariness and appetite regulation: pathways to obesity." *Clin Sci (Lond).* **130**, 1615-1628.

Hourihan, J.M., Kenna, J.G., and Hayes, J.D. (2013) "The gasotransmitter hydrogen sulfide induces nrf2-target genes by inactivating the keap1 ubiquitin ligase substrate adaptor through formation of a disulfide bond between cys-226 and cys-613." *Antioxid Redox Signal.* **19**, 465-481.

Hu, M.Z., Zhou, B., Mao, H.Y., Sheng, Q., Du, B., Chen, J.L., Pang, Q.F., and Ji, Y. (2016) "Exogenous Hydrogen Sulfide Postconditioning Protects Isolated Rat Hearts From Ischemia/Reperfusion Injury Through Sirt1/PGC-1α Signaling Pathway." *Int Heart J.* **57**, 477-482.

Hu, Q., Zhang, H., Gutiérrez Cortés, N., Wu, D., Wang, P., Zhang, J., Mattison, J.A., Smith, E., Bettcher, L.F., Wang, M., et al. (2020) "Increased Drp1 Acetylation by Lipid Overload Induces Cardiomyocyte Death and Heart Dysfunction." *Circ Res.* **126**, 456-470.

Ji, R., Akashi, H., Drosatos, K., Liao, X., Jiang, H., Kennel, P.J., Brunjes, D.L., Castillero, E., Zhang, X., Deng, L.Y., et al. (2017) "Increased de novo ceramide synthesis and accumulation in failing myocardium." *JCI Insight.* **2**.

Jiang, J., Chan, A., Ali, S., Saha, A., Haushalter, K.J., Lam, W.L., Glasheen, M., Parker, J., Brenner, M., Mahon, S.B., et al. (2016) "Hydrogen Sulfide--Mechanisms of Toxicity and Development of an Antidote." *Sci Rep.* **6**, 20831.

Jin, S., Teng, X., Xiao, L., Xue, H., Guo, Q., Duan, X., Chen, Y., and Wu, Y. (2017) "Hydrogen sulfide ameliorated L-NAME-induced hypertensive heart disease by the Akt/eNOS/NO pathway." *Exp Biol Med (Maywood).* **242**, 1831-1841.

Kabel, A.M. (2014) "Free Radicals and Antioxidants: Role of Enzymes and Nutrition." *World Journal of Nutrition and Health.* **2**, 35-38.

Kabil, O., and Banerjee, R. (2014) "Enzymology of H2S biogenesis, decay and signaling." *Antioxid Redox Signal.* **20**, 770-782.

Kabil, O., Motl, N., and Banerjee, R. (2014) "H2S and its role in redox signaling." *Biochim Biophys Acta.* **1844**, 1355-1366.

Kabil, O., Vitvitsky, V., Xie, P., and Banerjee, R. (2011) "The quantitative significance of the transsulfuration enzymes for H2S production in murine tissues." *Antioxid Redox Signal.* **15**, 363-372.

Kanaan, G.N., and Harper, M.E. (2017) "Cellular redox dysfunction in the development of cardiovascular diseases." *Biochim Biophys Acta Gen Subj.* **1861**, 2822-2829.

Kang, H., Jung, J.W., Kim, M.K., and Chung, J.H. (2009) "CK2 is the regulator of SIRT1 substrate-binding affinity, deacetylase activity and cellular response to DNA-damage." *PLoS One.* **4**, e6611.

Kang, Y.S. (2013) "Obesity associated hypertension: new insights into mechanism." *Electrolyte Blood Press.* **11**, 46-52.

Kar, S., Shahshahan, H.R., Hackfort, B.T., Yadav, S.K., Yadav, R., Kambis, T.N., Lefer, D.J., and Mishra, P.K. (2019) "Exercise training promotes cardiac hydrogen sulfide biosynthesis and mitigates pyroptosis to prevent high-fat diet-induced diabetic cardiomyopathy." *Antioxidants (Basel).* **8**.

Karam, B.S., Chavez-Moreno, A., Koh, W., Akar, J.G., and Akar, F.G. (2017) "Oxidative stress and inflammation as central mediators of atrial fibrillation in obesity and diabetes." *Cardiovasc Diabetol.* **16**, 120.

Kato, H., Han, X., Yamaza, H., Masuda, K., Hirofuji, Y., Sato, H., Pham, T.T.M., Taguchi, T., and Nonaka, K. (2017) "Direct effects of mitochondrial dysfunction on poor bone health in Leigh syndrome." *Biochem Biophys Res Commun.* **493**, 207-212.

Kimura, H. (2012) "Metabolic turnover of hydrogen sulfide." *Front Physiol.* **3**, 101.

Kimura, H. (2013) "Physiological role of hydrogen sulfide and polysulfide in the central nervous system." *Neurochem Int.* **63**, 492-497.

Kimura, H. (2020) "Signalling by hydrogen sulfide and polysulfides via protein S-sulfuration." *Br J Pharmacol.* **177**, 720-733.

Kitzman, D.W., and Shah, S.J. (2016) "The HFpEF Obesity Phenotype: The Elephant in the Room." *J Am Coll Cardiol.* **68**, 200-203.

Koliaki, C., Liatis, S., and Kokkinos, A. (2019) "Obesity and cardiovascular disease: revisiting an old relationship." *Metabolism.* **92**, 98-107.

Kolluru, G.K., Shen, X., Bir, S.C., and Kevil, C.G. (2013) "Hydrogen sulfide chemical biology: pathophysiological roles and detection." *Nitric Oxide.* **35**, 5-20.

Kondo, K., Bhushan, S., King, A.L., Prabhu, S.D., Hamid, T., Koenig, S., Murohara, T., Predmore, B.L., Gojon, G., Sr., Gojon, G., Jr., et al. (2013) "H_2 S protects against

pressure overload-induced heart failure via upregulation of endothelial nitric oxide synthase." *Circulation.* **127**, 1116-1127.

Kopelman, P.G. (2000) "Obesity as a medical problem." *Nature.* **404**, 635-643.

Kumar, P., Nagarajan, A., and Uchil, P.D. (2018) "Analysis of cell viability by the MTT assay." *Cold Spring Harb Protoc.* **2018**.

Kuschman, H.P., Palczewski, M.B., and Thomas, D.D. (2021) "Nitric oxide and hydrogen sulfide: Sibling rivalry in the family of epigenetic regulators." *Free Radic Biol Med.*

Lavu, M., Bhushan, S., and Lefer, D.J. (2011) "Hydrogen sulfide-mediated cardioprotection: mechanisms and therapeutic potential." *Clin Sci (Lond).* **120**, 219-229.

Lazarević, M., Mazzon, E., Momčilović, M., Basile, M.S., Colletti, G., Petralia, M.C., Bramanti, P., Nicoletti, F., and Miljković, Đ. (2018) "The H_2 S Donor GYY4137 Stimulates Reactive Oxygen Species Generation in BV2 Cells While Suppressing the Secretion of TNF and Nitric Oxide." *Molecules.* **23**.

Lee, B.Y., Han, J.A., Im, J.S., Morrone, A., Johung, K., Goodwin, E.C., Kleijer, W.J., DiMaio, D., and Hwang, E.S. (2006) "Senescence-associated beta-galactosidase is lysosomal beta-galactosidase." *Aging Cell.* **5**, 187-195.

Lee, H.Y., Lee, G.H., Bhattarai, K.R., Park, B.H., Koo, S.H., Kim, H.R., and Chae, H.J. (2016) "Bax Inhibitor-1 regulates hepatic lipid accumulation via ApoB secretion." *Sci Rep.* **6**, 27799.

Lee, J., Yamaoka, Y., Kong, F., Cagnon, C., Beyly-Adriano, A., Jang, S., Gao, P., Kang, B.H., Li-Beisson, Y., and Lee, Y. (2020) "The phosphatidylethanolamine-binding protein DTH1 mediates degradation of lipid droplets in Chlamydomonas reinhardtii." *Proc Natl Acad Sci U S A.* **117**, 23131-23139.

Leslie, M. (2008) "Medicine. Nothing rotten about hydrogen sulfide's medical promise." *Science.* **320**, 1155-1157.

Levine, A.J. (2019) "The many faces of p53: something for everyone." *J Mol Cell Biol.* **11**, 524-530.

Li, H., Fan, J., Zhao, Y., Zhang, X., Dai, B., Zhan, J., Yin, Z., Nie, X., Fu, X.D., Chen, C., et al. (2019) "Nuclear miR-320 Mediates Diabetes-Induced Cardiac Dysfunction by Activating Transcription of Fatty Acid Metabolic Genes to Cause Lipotoxicity in the Heart." *Circ Res.* **125**, 1106-1120.

Li, L.O., Klett, E.L., and Coleman, R.A. (2010) "Acyl-CoA synthesis, lipid metabolism and lipotoxicity." *Biochim Biophys Acta.* **1801**, 246-251.

Li, Y., Li, M., Shi, J., Yang, X., and Wang, Z. (2012) "Hepatic antioxidative responses to PCDPSs and estimated short-term biotoxicity in freshwater fish." *Aquat Toxicol.* **120-121**, 90-98.

Libiad, M., Vitvitsky, V., Bostelaar, T., Bak, D.W., Lee, H.J., Sakamoto, N., Fearon, E., Lyssiotis, C.A., Weerapana, E., and Banerjee, R. (2019) "Hydrogen sulfide perturbs

mitochondrial bioenergetics and triggers metabolic reprogramming in colon cells." *J Biol Chem.* **294**, 12077-12090.

Lin, Y.H., Schmidt, W., Fritz, K.S., Jeong, M.Y., Cammarato, A., Foster, D.B., Biesiadecki, B.J., McKinsey, T.A., and Woulfe, K.C. (2020) "Site-specific acetyl-mimetic modification of cardiac troponin I modulates myofilament relaxation and calcium sensitivity." *J Mol Cell Cardiol.* **139**, 135-147.

Linden, D.R., Levitt, M.D., Farrugia, G., and Szurszewski, J.H. (2010) "Endogenous production of H_2S in the gastrointestinal tract: still in search of a physiologic function." *Antioxid Redox Signal.* **12**, 1135-1146.

Liu, M., Chen, F., Liu, T., Chen, F., Liu, S., and Yang, J. (2017) "The role of oxidative stress in influenza virus infection." *Microbes Infect.* **19**, 580-586.

Lkhagva, B., Kao, Y.H., Lee, T.I., Lee, T.W., Cheng, W.L., and Chen, Y.J. (2018) "Activation of Class I histone deacetylases contributes to mitochondrial dysfunction in cardiomyocytes with altered complex activities." *Epigenetics.* **13**, 376-385.

Lopaschuk, G.D., Collins-Nakai, R., Olley, P.M., Montague, T.J., McNeil, G., Gayle, M., Penkoske, P., and Finegan, B.A. (1994) "Plasma fatty acid levels in infants and adults after myocardial ischemia." *Am Heart J.* **128**, 61-67.

Lopaschuk, G.D., Folmes, C.D., and Stanley, W.C. (2007) "Cardiac energy metabolism in obesity." *Circ Res.* **101**, 335-347.

Lopaschuk, G.D., Ussher, J.R., Folmes, C.D., Jaswal, J.S., and Stanley, W.C. (2010) "Myocardial fatty acid metabolism in health and disease." *Physiol Rev.* **90**, 207-258.

Łuczaj, W., Gęgotek, A., and Skrzydlewska, E. (2017) "Antioxidants and HNE in redox homeostasis." *Free Radic Biol Med.* **111**, 87-101.

Luiken, J.J., Arumugam, Y., Dyck, D.J., Bell, R.C., Pelsers, M.M., Turcotte, L.P., Tandon, N.N., Glatz, J.F., and Bonen, A. (2001) "Increased rates of fatty acid uptake and plasmalemmal fatty acid transporters in obese Zucker rats." *J Biol Chem.* **276**, 40567-40573.

Luo, W., Gui, D.D., Yan, B.J., Ren, Z., Peng, L.J., Wei, D.H., Liu, L.S., Zhang, D.W., and Jiang, Z.S. (2020) "Hydrogen Sulfide Switch Phenomenon Regulating Autophagy in Cardiovascular Diseases." *Cardiovasc Drugs Ther.* **34**, 113-121.

Ma, P., and Schultz, R.M. (2008) "Histone deacetylase 1 (HDAC1) regulates histone acetylation, development, and gene expression in preimplantation mouse embryos." *Dev Biol.* **319**, 110-120.

MacLean, P.S., Blundell, J.E., Mennella, J.A., and Batterham, R.L. (2017) "Biological control of appetite: A daunting complexity." *Obesity (Silver Spring).* **25 Suppl 1**, S8-s16.

Maeder, M.T., Thompson, B.R., Brunner-La Rocca, H.P., and Kaye, D.M. (2010) "Hemodynamic basis of exercise limitation in patients with heart failure and normal ejection fraction." *J Am Coll Cardiol.* **56**, 855-863.

Makrecka-Kuka, M., Liepinsh, E., Murray, A.J., Lemieux, H., Dambrova, M., Tepp, K., Puurand, M., Käämbre, T., Han, W.H., de Goede, P., et al. (2020) "Altered mitochondrial metabolism in the insulin-resistant heart." *Acta Physiol (Oxf).* **228**, e13430.

Mani, S., Li, H., Yang, G., Wu, L., and Wang, R. (2015) "Deficiency of cystathionine gamma-lyase and hepatic cholesterol accumulation during mouse fatty liver development." *Science Bulletin.* **60**, 336-347.

Manna, P., Gungor, N., McVie, R., and Jain, S.K. (2014) "Decreased cystathionine-γ-lyase (CSE) activity in livers of type 1 diabetic rats and peripheral blood mononuclear cells (PBMC) of type 1 diabetic patients." *J Biol Chem.* **289**, 11767-11778.

Martin, G.R., McKnight, G.W., Dicay, M.S., Coffin, C.S., Ferraz, J.G., and Wallace, J.L. (2010) "Hydrogen sulphide synthesis in the rat and mouse gastrointestinal tract." *Dig Liver Dis.* **42**, 103-109.

Matey-Hernandez, M.L., Williams, F.M.K., Potter, T., Valdes, A.M., Spector, T.D., and Menni, C. (2018) "Genetic and microbiome influence on lipid metabolism and dyslipidemia." *Physiol Genomics.* **50**, 117-126.

McGavock, J.M., Victor, R.G., Unger, R.H., and Szczepaniak, L.S. (2006) "Adiposity of the heart, revisited." *Ann Intern Med.* **144**, 517-524.

Meng, G., Xiao, Y., Ma, Y., Tang, X., Xie, L., Liu, J., Gu, Y., Yu, Y., Park, C.M., Xian, M., et al. (2016) "Hydrogen Sulfide Regulates Krüppel-Like Factor 5 Transcription Activity

via Specificity Protein 1 S-Sulfhydration at Cys664 to Prevent Myocardial Hypertrophy." *J Am Heart Assoc.* **5**.

Mews, P., Donahue, G., Drake, A.M., Luczak, V., Abel, T., and Berger, S.L. (2017) "Acetyl-CoA synthetase regulates histone acetylation and hippocampal memory." *Nature.* **546**, 381-386.

Mobley, R.J., Raghu, D., Duke, L.D., Abell-Hart, K., Zawistowski, J.S., Lutz, K., Gomez, S.M., Roy, S., Homayouni, R., Johnson, G.L., et al. (2017) "MAP3K4 controls the chromatin modifier HDAC6 during trophoblast stem cell epithelial-to-mesenchymal transition." *Cell Rep.* **18**, 2387-2400.

Módis, K., Ju, Y., Ahmad, A., Untereiner, A.A., Altaany, Z., Wu, L., Szabo, C., and Wang, R. (2016) "S-Sulfhydration of ATP synthase by hydrogen sulfide stimulates mitochondrial bioenergetics." *Pharmacol Res.* **113**, 116-124.

Moldogazieva, N.T., Mokhosoev, I.M., Feldman, N.B., and Lutsenko, S.V. (2018) "ROS and RNS signalling: adaptive redox switches through oxidative/nitrosative protein modifications." *Free Radic Res.* **52**, 507-543.

Moldogazieva, N.T., Mokhosoev, I.M., Mel'nikova, T.I., Porozov, Y.B., and Terentiev, A.A. (2019) "Oxidative stress and advanced lipoxidation and glycation end products (ALEs and AGEs) in aging and age-related diseases." *Oxid Med Cell Longev.* **2019**, 3085756.

Monji, A., Mitsui, T., Bando, Y.K., Aoyama, M., Shigeta, T., and Murohara, T. (2013) "Glucagon-like peptide-1 receptor activation reverses cardiac remodeling via normalizing cardiac steatosis and oxidative stress in type 2 diabetes." *Am J Physiol Heart Circ Physiol.* **305**, H295-304.

Morales, C.R., Li, D.L., Pedrozo, Z., May, H.I., Jiang, N., Kyrychenko, V., Cho, G.W., Kim, S.Y., Wang, Z.V., Rotter, D., et al. (2016) "Inhibition of class I histone deacetylases blunts cardiac hypertrophy through TSC2-dependent mTOR repression." *Sci Signal.* **9**, ra34.

Nakatani, K., Masuda, D., Kobayashi, T., Sairyo, M., Zhu, Y., Okada, T., Naito, A.T., Ohama, T., Koseki, M., Oka, T., et al. (2019) "Pressure Overload Impairs Cardiac Function in Long-Chain Fatty Acid Transporter CD36-Knockout Mice." *Int Heart J.* **60**, 159-167.

Nielsen, L.B., Bartels, E.D., and Bollano, E. (2002a) "Overexpression of apolipoprotein B in the heart impedes cardiac triglyceride accumulation and development of cardiac dysfunction in diabetic mice." *J Biol Chem.* **277**, 27014-27020.

Nielsen, L.B., Perko, M., Arendrup, H., and Andersen, C.B. (2002b) "Microsomal triglyceride transfer protein gene expression and triglyceride accumulation in hypoxic human hearts." *Arterioscler Thromb Vasc Biol.* **22**, 1489-1494.

Niki, E. (2016) "Oxidative stress and antioxidants: Distress or eustress?" *Arch Biochem Biophys.* **595**, 19-24.

Normandin, E., Houston, D.K., and Nicklas, B.J. (2015) "Caloric restriction for treatment of geriatric obesity: Do the benefits outweigh the risks?" *Curr Nutr Rep.* **4**, 143-155.

Nural-Guvener, H.F., Zakharova, L., Nimlos, J., Popovic, S., Mastroeni, D., and Gaballa, M.A. (2014) "HDAC class I inhibitor, Mocetinostat, reverses cardiac fibrosis in heart failure and diminishes CD90+ cardiac myofibroblast activation." *Fibrogenesis Tissue Repair.* **7**, 10.

Obokata, M., Reddy, Y.N.V., Pislaru, S.V., Melenovsky, V., and Borlaug, B.A. (2017) "Evidence Supporting the Existence of a Distinct Obese Phenotype of Heart Failure With Preserved Ejection Fraction." *Circulation.* **136**, 6-19.

Orimo, M., Minamino, T., Miyauchi, H., Tateno, K., Okada, S., Moriya, J., and Komuro, I. (2009) "Protective role of SIRT1 in diabetic vascular dysfunction." *Arterioscler Thromb Vasc Biol.* **29**, 889-894.

Ota, H., Akishita, M., Eto, M., Iijima, K., Kaneki, M., and Ouchi, Y. (2007) "Sirt1 modulates premature senescence-like phenotype in human endothelial cells." *J Mol Cell Cardiol.* **43**, 571-579.

Pan, T.T., Feng, Z.N., Lee, S.W., Moore, P.K., and Bian, J.S. (2006) "Endogenous hydrogen sulfide contributes to the cardioprotection by metabolic inhibition preconditioning in the rat ventricular myocytes." *J Mol Cell Cardiol.* **40**, 119-130.

Panthi, S., Chung, H.J., Jung, J., and Jeong, N.Y. (2016) "Physiological importance of hydrogen sulfide: Emerging potent neuroprotector and neuromodulator." *Oxid Med Cell Longev.* **2016**, 9049782.

Papa, S. (1996) "Mitochondrial oxidative phosphorylation changes in the life span. Molecular aspects and physiopathological implications." *Biochim Biophys Acta.* **1276**, 87-105.

Parisi, V., Rengo, G., Perrone-Filardi, P., Pagano, G., Femminella, G.D., Paolillo, S., Petraglia, L., Gambino, G., Caruso, A., Grimaldi, M.G., et al. (2016) "Increased Epicardial Adipose Tissue Volume Correlates With Cardiac Sympathetic Denervation in Patients With Heart Failure." *Circ Res.* **118**, 1244-1253.

Park, T.S., and Goldberg, I.J. (2012) "Sphingolipids, lipotoxic cardiomyopathy, and cardiac failure." *Heart Fail Clin.* **8**, 633-641.

Parrillo, L., Costa, V., Raciti, G.A., Longo, M., Spinelli, R., Esposito, R., Nigro, C., Vastolo, V., Desiderio, A., Zatterale, F., et al. (2016) "Hoxa5 undergoes dynamic DNA methylation and transcriptional repression in the adipose tissue of mice exposed to high-fat diet." *Int J Obes (Lond).* **40**, 929-937.

Patel, P., Vatish, M., Heptinstall, J., Wang, R., and Carson, R.J. (2009) "The endogenous production of hydrogen sulphide in intrauterine tissues." *Reprod Biol Endocrinol.* **7**, 10.

Paulus, W.J., and Tschöpe, C. (2013) "A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation." *J Am Coll Cardiol.* **62**, 263-271.

Perridon, B.W., Leuvenink, H.G., Hillebrands, J.L., van Goor, H., and Bos, E.M. (2016) "The role of hydrogen sulfide in aging and age-related pathologies." *Aging (Albany NY).* **8**, 2264-2289.

Perrin, S., Cremer, J., Roll, P., Faucher, O., Ménard, A., Reynes, J., Dellamonica, P., Naqvi, A., Micallef, J., Jouve, E., et al. (2012) "HIV-1 infection and first line ART induced differential responses in mitochondria from blood lymphocytes and monocytes: the ANRS EP45 "Aging" study." *PLoS One.* **7**, e41129.

Pinkerneil, M., Hoffmann, M.J., Deenen, R., Köhrer, K., Arent, T., Schulz, W.A., and Niegisch, G. (2016) "Inhibition of Class I Histone Deacetylases 1 and 2 Promotes Urothelial Carcinoma Cell Death by Various Mechanisms." *Mol Cancer Ther.* **15**, 299-312.

Powell, C.R., Dillon, K.M., and Matson, J.B. (2018) "A review of hydrogen sulfide (H(2)S) donors: Chemistry and potential therapeutic applications." *Biochem Pharmacol.* **149**, 110-123.

Pryor, W.A., Houk, K.N., Foote, C.S., Fukuto, J.M., Ignarro, L.J., Squadrito, G.L., and Davies, K.J. (2006) "Free radical biology and medicine: it's a gas, man!" *Am J Physiol Regul Integr Comp Physiol.* **291**, R491-511.

Que, X., Hung, M.Y., Yeang, C., Gonen, A., Prohaska, T.A., Sun, X., Diehl, C., Määttä, A., Gaddis, D.E., Bowden, K., et al. (2018) "Oxidized phospholipids are proinflammatory and proatherogenic in hypercholesterolaemic mice." *Nature.* **558**, 301-306.

Raciti, G.A., Spinelli, R., Desiderio, A., Longo, M., Parrillo, L., Nigro, C., D'Esposito, V., Mirra, P., Fiory, F., Pilone, V., et al. (2017) "Specific CpG hyper-methylation leads to Ankrd26 gene down-regulation in white adipose tissue of a mouse model of diet-induced obesity." *Sci Rep.* **7**, 43526.

Rannikko, E.H., Vesterager, L.B., Shaik, J.H., Weber, S.S., Cornejo Castro, E.M., Fog, K., Jensen, P.H., and Kahle, P.J. (2013) "Loss of DJ-1 protein stability and cytoprotective function by Parkinson's disease-associated proline-158 deletion." *J Neurochem.* **125**, 314-327.

Rasola, A., and Bernardi, P. (2007) "The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis." *Apoptosis.* **12**, 815-833.

Rezayian, M., Niknam, V., and Ebrahimzadeh, H. (2019) "Oxidative damage and antioxidative system in algae." *Toxicol Rep.* **6**, 1309-1313.

Riehle, C., and Abel, E.D. (2016) "Insulin Signaling and Heart Failure." *Circ Res.* **118**, 1151-1169.

Rufini, A., Tucci, P., Celardo, I., and Melino, G. (2013) "Senescence and aging: the critical roles of p53." *Oncogene.* **32**, 5129-5143.

San Martín, A., Du, P., Dikalova, A., Lassègue, B., Aleman, M., Góngora, M.C., Brown, K., Joseph, G., Harrison, D.G., Taylor, W.R., et al. (2007) "Reactive oxygen species-selective regulation of aortic inflammatory gene expression in Type 2 diabetes." *Am J Physiol Heart Circ Physiol.* **292**, H2073-2082.

Sasaki, T., Maier, B., Bartke, A., and Scrable, H. (2006) "Progressive loss of SIRT1 with cell cycle withdrawal." *Aging Cell.* **5**, 413-422.

Sbodio, J.I., Snyder, S.H., and Paul, B.D. (2019) "Regulators of the transsulfuration pathway." *Br J Pharmacol.* **176**, 583-593.

Scher, M.B., Vaquero, A., and Reinberg, D. (2007) "SirT3 is a nuclear NAD+-dependent histone deacetylase that translocates to the mitochondria upon cellular stress." *Genes Dev.* **21**, 920-928.

Schulze, P.C., Drosatos, K., and Goldberg, I.J. (2016) "Lipid Use and Misuse by the Heart." *Circ Res.* **118**, 1736-1751.

Schwartz, M.W., Seeley, R.J., Zeltser, L.M., Drewnowski, A., Ravussin, E., Redman, L.M., and Leibel, R.L. (2017) "Obesity Pathogenesis: An Endocrine Society Scientific Statement." *Endocr Rev.* **38**, 267-296.

Sen, N. (2017) "Functional and Molecular Insights of Hydrogen Sulfide Signaling and Protein Sulfhydration." *J Mol Biol.* **429**, 543-561.

Seto, E., and Yoshida, M. (2014) "Erasers of histone acetylation: the histone deacetylase enzymes." *Cold Spring Harb Perspect Biol.* **6**, a018713.

Severino, J., Allen, R.G., Balin, S., Balin, A., and Cristofalo, V.J. (2000) "Is beta-galactosidase staining a marker of senescence in vitro and in vivo?" *Exp Cell Res.* **257**, 162-171.

Shah, S.J., Kitzman, D.W., Borlaug, B.A., van Heerebeek, L., Zile, M.R., Kass, D.A., and Paulus, W.J. (2016) "Phenotype-Specific Treatment of Heart Failure With Preserved Ejection Fraction: A Multiorgan Roadmap." *Circulation.* **134**, 73-90.

Shaw, B.F., Schneider, G.F., Bilgiçer, B., Kaufman, G.K., Neveu, J.M., Lane, W.S., Whitelegge, J.P., and Whitesides, G.M. (2008) "Lysine acetylation can generate highly charged enzymes with increased resistance toward irreversible inactivation." *Protein Sci.* **17**, 1446-1455.

Shen, Y., Shen, Z., Luo, S., Guo, W., and Zhu, Y.Z. (2015) "The cardioprotective effects of hydrogen sulfide in heart diseases: From molecular mechanisms to therapeutic potential." *Oxid Med Cell Longev.* **2015**, 925167.

Shi, L., and Tu, B.P. (2015) "Acetyl-CoA and the regulation of metabolism: mechanisms and consequences." *Curr Opin Cell Biol.* **33**, 125-131.

Shibuya, N., Koike, S., Tanaka, M., Ishigami-Yuasa, M., Kimura, Y., Ogasawara, Y., Fukui, K., Nagahara, N., and Kimura, H. (2013) "A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells." *Nat Commun.* **4**, 1366.

Shibuya, N., Tanaka, M., Yoshida, M., Ogasawara, Y., Togawa, T., Ishii, K., and Kimura, H. (2009) "3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain." *Antioxid Redox Signal.* **11**, 703-714.

Shimizu, I., and Minamino, T. (2019) "Cellular senescence in cardiac diseases." *J Cardiol.* **74**, 313-319.

Singh, A.K., Bishayee, A., and Pandey, A.K. (2018) "Targeting Histone Deacetylases with Natural and Synthetic Agents: An Emerging Anticancer Strategy." *Nutrients.* **10**.

Sletten, A.C., Peterson, L.R., and Schaffer, J.E. (2018) "Manifestations and mechanisms of myocardial lipotoxicity in obesity." *J Intern Med.* **284**, 478-491.

Sokolov, A.S., Nekrasov, P.V., Shaposhnikov, M.V., and Moskalev, A.A. (2021) "Hydrogen sulfide in longevity and pathologies: Inconsistency is malodorous." *Ageing Res Rev.* **67**, 101262.

Stockert, J.C., Blázquez-Castro, A., Cañete, M., Horobin, R.W., and Villanueva, A. (2012) "MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets." *Acta Histochem.* **114**, 785-796.

Su, L.J., Zhang, J.H., Gomez, H., Murugan, R., Hong, X., Xu, D., Jiang, F., and Peng, Z.Y. (2019) "Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis." *Oxid Med Cell Longev.* **2019**, 5080843.

Sun, L., Zhang, S., Yu, C., Pan, Z., Liu, Y., Zhao, J., Wang, X., Yun, F., Zhao, H., Yan, S., et al. (2015) "Hydrogen sulfide reduces serum triglyceride by activating liver autophagy via the AMPK-mTOR pathway." *Am J Physiol Endocrinol Metab.* **309**, E925-935.

Sun, Y., Teng, Z., Sun, X., Zhang, L., Chen, J., Wang, B., Lu, F., Liu, N., Yu, M., Peng, S., et al. (2019) "Exogenous H(2)S reduces the acetylation levels of mitochondrial respiratory enzymes via regulating the NAD(+)-SIRT3 pathway in cardiac tissues of db/db mice." *Am J Physiol Endocrinol Metab.* **317**, E284-e297.

Sverdlov, A.L., Elezaby, A., Qin, F., Behring, J.B., Luptak, I., Calamaras, T.D., Siwik, D.A., Miller, E.J., Liesa, M., Shirihai, O.S., et al. (2016) "Mitochondrial Reactive Oxygen Species Mediate Cardiac Structural, Functional, and Mitochondrial Consequences of Diet-Induced Metabolic Heart Disease." *J Am Heart Assoc.* **5**.

Szabo, C., Coletta, C., Chao, C., Módis, K., Szczesny, B., Papapetropoulos, A., and Hellmich, M.R. (2013) "Tumor-derived hydrogen sulfide, produced by cystathionine-β-synthase, stimulates bioenergetics, cell proliferation, and angiogenesis in colon cancer." *Proc Natl Acad Sci U S A.* **110**, 12474-12479.

Szabo, C., and Papapetropoulos, A. (2017) "International Union of Basic and Clinical Pharmacology. CII: Pharmacological Modulation of H(2)S Levels: H(2)S Donors and H(2)S Biosynthesis Inhibitors." *Pharmacol Rev.* **69**, 497-564.

Teng, H., Wu, B., Zhao, K., Yang, G., Wu, L., and Wang, R. (2013) "Oxygen-sensitive mitochondrial accumulation of cystathionine β-synthase mediated by Lon protease." *Proc Natl Acad Sci U S A.* **110**, 12679-12684.

Todoric, J., Di Caro, G., Reibe, S., Henstridge, D.C., Green, C.R., Vrbanac, A., Ceteci, F., Conche, C., McNulty, R., Shalapour, S., et al. (2020) "Fructose stimulated de novo lipogenesis is promoted by inflammation." *Nat Metab.* **2**, 1034-1045.

Tomita, M., Nagahara, N., and Ito, T. (2016) "Expression of 3-Mercaptopyruvate Sulfurtransferase in the Mouse." *Molecules.* **21**.

Topf, U., Wrobel, L., and Chacinska, A. (2016) "Chatty mitochondria: Keeping balance in cellular protein homeostasis." *Trends Cell Biol.* **26**, 577-586.

Truong, D.H., Eghbal, M.A., Hindmarsh, W., Roth, S.H., and O'Brien, P.J. (2006) "Molecular mechanisms of hydrogen sulfide toxicity." *Drug Metab Rev.* **38**, 733-744.

Untergasser, G., Gander, R., Rumpold, H., Heinrich, E., Plas, E., and Berger, P. (2003) "TGF-beta cytokines increase senescence-associated beta-galactosidase activity in human prostate basal cells by supporting differentiation processes, but not cellular senescence." *Exp Gerontol.* **38**, 1179-1188.

Upadhya, B., Haykowsky, M.J., Eggebeen, J., and Kitzman, D.W. (2015) "Sarcopenic obesity and the pathogenesis of exercise intolerance in heart failure with preserved ejection fraction." *Curr Heart Fail Rep.* **12**, 205-214.

Vakifahmetoglu-Norberg, H., Ouchida, A.T., and Norberg, E. (2017) "The role of mitochondria in metabolism and cell death." *Biochem Biophys Res Commun.* **482**, 426-431.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., and Telser, J. (2007) "Free radicals and antioxidants in normal physiological functions and human disease." *Int J Biochem Cell Biol.* **39**, 44-84.

van de Weijer, T., Schrauwen-Hinderling, V.B., and Schrauwen, P. (2011) "Lipotoxicity in type 2 diabetic cardiomyopathy." *Cardiovasc Res.* **92**, 10-18.

Vega, R.B., and Kelly, D.P. (2017) "Cardiac nuclear receptors: architects of mitochondrial structure and function." *J Clin Invest.* **127**, 1155-1164.

Venardos, K.M., Perkins, A., Headrick, J., and Kaye, D.M. (2007) "Myocardial ischemia-reperfusion injury, antioxidant enzyme systems, and selenium: a review." *Curr Med Chem.* **14**, 1539-1549.

Vieira, G.L.T., Lossie, A.C., Lay, D.C., Jr., Radcliffe, J.S., and Garner, J.P. (2017) "Preventing, treating, and predicting barbering: A fundamental role for biomarkers of oxidative stress in a mouse model of Trichotillomania." *PLoS One.* **12**, e0175222.

Wakabayashi, H., Maeda, K., Nishioka, S., Shamoto, H., and Momosaki, R. (2019) "Impact of body mass index on activities of daily living in inpatients with acute heart failure." *J Nutr Health Aging.* **23**, 151-156.

Wallner, M., Eaton, D.M., Berretta, R.M., Liesinger, L., Schittmayer, M., Gindlhuber, J., Wu, J., Jeong, M.Y., Lin, Y.H., Borghetti, G., et al. (2020) "HDAC inhibition improves cardiopulmonary function in a feline model of diastolic dysfunction." *Sci Transl Med.* **12**.

Wang, R. (2012) "Physiological implications of hydrogen sulfide: a whiff exploration that blossomed." *Physiol Rev.* **92**, 791-896.

Wang, Y., Gao, P., Wei, C., Li, H., Zhang, L., Zhao, Y., Wu, B., Tian, Y., Zhang, W., Wu, L., et al. (2017) "Calcium sensing receptor protects high glucose-induced energy metabolism disorder via blocking gp78-ubiquitin proteasome pathway." *Cell Death Dis.* **8**, e2799.

Wang, Y., Yu, R., Wu, L., and Yang, G. (2020) "Hydrogen sulfide signaling in regulation of cell behaviors." *Nitric Oxide.* **103**, 9-19.

Wang, Y., Yu, R., Wu, L., and Yang, G. (2021) "Hydrogen sulfide guards myoblasts from ferroptosis by inhibiting ALOX12 acetylation." *Cell Signal.* **78**, 109870.

Wende, A.R., and Abel, E.D. (2010) "Lipotoxicity in the heart." *Biochim Biophys Acta.* **1801**, 311-319.

Wende, A.R., Symons, J.D., and Abel, E.D. (2012) "Mechanisms of lipotoxicity in the cardiovascular system." *Curr Hypertens Rep.* **14**, 517-531.

Wong, C., and Marwick, T.H. (2007) "Obesity cardiomyopathy: pathogenesis and pathophysiology." *Nat Clin Pract Cardiovasc Med.* **4**, 436-443.

Wu, D., Hu, Q., Liu, X., Pan, L., Xiong, Q., and Zhu, Y.Z. (2015) "Hydrogen sulfide protects against apoptosis under oxidative stress through SIRT1 pathway in H9c2 cardiomyocytes." *Nitric Oxide.* **46**, 204-212.

Wu, D., Wang, H., Teng, T., Duan, S., Ji, A., and Li, Y. (2018) "Hydrogen sulfide and autophagy: A double edged sword." *Pharmacol Res.* **131**, 120-127.

Wu, K.M., Hsu, Y.M., Ying, M.C., Tsai, F.J., Tsai, C.H., Chung, J.G., Yang, J.S., Tang, C.H., Cheng, L.Y., Su, P.H., et al. (2019) "High-density lipoprotein ameliorates palmitic acid-induced lipotoxicity and oxidative dysfunction in H9c2 cardiomyoblast cells via ROS suppression." *Nutr Metab (Lond).* **16**, 36.

Xiao, A.Y., Maynard, M.R., Piett, C.G., Nagel, Z.D., Alexander, J.S., Kevil, C.G., Berridge, M.V., Pattillo, C.B., Rosen, L.R., Miriyala, S., et al. (2019) "Sodium sulfide selectively induces oxidative stress, DNA damage, and mitochondrial dysfunction and radiosensitizes glioblastoma (GBM) cells." *Redox Biol.* **26**, 101220.

Xie, Y.H., Zhang, N., Li, L.F., Zhang, Q.Z., Xie, L.J., Jiang, H., Li, L.P., Hao, N., and Zhang, J.X. (2014a) "Hydrogen sulfide reduces regional myocardial ischemia injury through protection of mitochondrial function." *Mol Med Rep.* **10**, 1907-1914.

Xie, Z.Z., Shi, M.M., Xie, L., Wu, Z.Y., Li, G., Hua, F., and Bian, J.S. (2014b) "Sulfhydration of p66Shc at cysteine59 mediates the antioxidant effect of hydrogen sulfide." *Antioxid Redox Signal.* **21**, 2531-2542.

Xin, H., Wang, M., Tang, W., Shen, Z., Miao, L., Wu, W., Li, C., Wang, X., Xin, X., and Zhu, Y.Z. (2016) "Hydrogen Sulfide Attenuates Inflammatory Hepcidin by Reducing IL-6 Secretion and Promoting SIRT1-Mediated STAT3 Deacetylation." *Antioxid Redox Signal.* **24**, 70-83.

Xiong, Y., and Guan, K.L. (2012) "Mechanistic insights into the regulation of metabolic enzymes by acetylation." *J Cell Biol.* **198**, 155-164.

Xu, Y., Dai, X., Zhu, D., Xu, X., Gao, C., and Wu, C. (2015) "An exogenous hydrogen sulphide donor, NaHS, inhibits the apoptosis signaling pathway to exert cardio-protective effects in a rat hemorrhagic shock model." *Int J Clin Exp Pathol.* **8**, 6245-6254.

Yang, G., Ju, Y., Fu, M., Zhang, Y., Pei, Y., Racine, M., Baath, S., Merritt, T.J.S., Wang, R., and Wu, L. (2018) "Cystathionine gamma-lyase/hydrogen sulfide system is essential for adipogenesis and fat mass accumulation in mice." *Biochim Biophys Acta Mol Cell Biol Lipids.* **1863**, 165-176.

Yang, G., Wu, L., and Wang, R. (2006) "Pro-apoptotic effect of endogenous H2S on human aorta smooth muscle cells." *Faseb j.* **20**, 553-555.

Yang, J., He, J., Ismail, M., Tweeten, S., Zeng, F., Gao, L., Ballinger, S., Young, M., Prabhu, S.D., Rowe, G.C., et al. (2019) "HDAC inhibition induces autophagy and mitochondrial biogenesis to maintain mitochondrial homeostasis during cardiac ischemia/reperfusion injury." *J Mol Cell Cardiol.* **130**, 36-48.

Yang, Q., and He, G.W. (2019) "Imbalance of Homocysteine and H(2)S: Significance, Mechanisms, and Therapeutic Promise in Vascular Injury." *Oxid Med Cell Longev.* **2019**, 7629673.

Yang, W., Yang, G., Jia, X., Wu, L., and Wang, R. (2005) "Activation of KATP channels by H_2S in rat insulin-secreting cells and the underlying mechanisms." *J Physiol.* **569**, 519-531.

Yazıcı, D., and Sezer, H. (2017) "Insulin resistance, obesity and lipotoxicity." *Adv Exp Med Biol.* **960**, 277-304.

Yeo, D., Kang, C., and Ji, L.L. (2020) "Aging alters acetylation status in skeletal and cardiac muscles." *Geroscience.* **42**, 963-976.

Yi, M., Li, J., Chen, S., Cai, J., Ban, Y., Peng, Q., Zhou, Y., Zeng, Z., Peng, S., Li, X., et al. (2018) "Emerging role of lipid metabolism alterations in cancer stem cells." *J Exp Clin Cancer Res.* **37**, 118.

Young, M.E., Guthrie, P.H., Razeghi, P., Leighton, B., Abbasi, S., Patil, S., Youker, K.A., and Taegtmeyer, H. (2002) "Impaired long-chain fatty acid oxidation and contractile dysfunction in the obese Zucker rat heart." *Diabetes.* **51**, 2587-2595.

Zhang, H., Zhang, X., Li, X., Meng, W.B., Bai, Z.T., Rui, S.Z., Wang, Z.F., Zhou, W.C., and Jin, X.D. (2018) "Effect of CCNB1 silencing on cell cycle, senescence, and apoptosis through the p53 signaling pathway in pancreatic cancer." *J Cell Physiol.* **234**, 619-631.

Zhang, J., Sprung, R., Pei, J., Tan, X., Kim, S., Zhu, H., Liu, C.F., Grishin, N.V., and Zhao, Y. (2009) "Lysine acetylation is a highly abundant and evolutionarily conserved modification in Escherichia coli." *Mol Cell Proteomics.* **8**, 215-225.

Zhang, Y.E., Huang, G.Q., Wu, B., Lin, X.D., Yang, W.Z., Ke, Z.Y., and Liu, J. (2019) "Hydrogen sulfide protects H9c2 cardiomyoblasts against H2O2-induced apoptosis." *Braz J Med Biol Res.* **52**, e7626.

Zhao, W., Zhang, J., Lu, Y., and Wang, R. (2001) "The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener." *EMBO J.* **20**, 6008-6016.