Role of chaperones and endoplasmic reticulum stress in protein complexity associated with dyslipidemia: A future perspective to novel therapeutics (Review)

SUHAIB AL HUQ MOHAMMED[1] and KIRUBA RAJAMANI[2]

[1]Electronics and Communication Engineering, School of Engineering, Amrita Vishwa Vidyapeetham, Tiruvallur,Tamil Nadu 601103; [2]Department of Biotechnology, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu 641002, India

Received September 12, 2023; Accepted November 28, 2023

DOI: 10.3892/wasj.2023.218

Abstract. Protein structure is extraordinarily complex and consists of various folding processes, namely, primary, secondary, tertiary and quaternary. Currently, researchers are eager to acquire knowledge and elucidate the mechanisms involved in these processes. Over the past decade, several studies have indicated that the endoplasmic reticulum stress (ERS) response assists in the protein response via intracellular signaling. ERS plays a key role in protein folding and unfolding. An increase in ERS promotes the unwinding of the folded protein. The present review specifically discusses the protein complexity involved in dyslipidemia (the condition of elevated cholesterol levels in the blood) with the ERS response and unfolded protein response. ERS in dyslipidemia is achieved by several pathological conditions, including oxidative stress, calcium imbalance and high levels of cholesterol in the blood. Therefore, in the present review, the ERS response in dyslipidemia is specifically highlighted as regards protein complexity, which indicates that considering oxidative stress, calcium imbalance and high levels of cholesterol would be a great benefit to reducing the ERS. The present literature review was performed using the search engines of the web-based databases (Google Scholar and ScienceDirect) with keywords such as 'Role of chaperones in dyslipidemia' and 'Protein complexity' or 'Protein involved in dyslipidemia'. Furthermore, the role of chaperones associated with ER in finding novel therapeutics for disease prevention is also discussed.

Correspondence to: Professor Kiruba Rajamani, Department of Biotechnology, PSGR Krishnammal College for Women, Avinashi Road, Peelamedu, Coimbatore, Tamil Nadu 641002, India
E-mail: kirubadevi.r@gmail.com

Abbreviations: ER, endoplasmic reticulum; CVD, cardiovascular disease; HSP, heat shock protein; PCSK9, proprotein convertase subtilisin/kexin type 9; LXR, liver X receptor; S1P, sphingosine-1-phosphate; PPAR, peroxisome proliferator-activated receptor; NAFLD, non-alcoholic fatty liver disease; GRP, glucose regulated protein; ERAD, endoplasmic reticulum-associated protein degradation; IRE1, inositol requiring enzyme 1; PERK, protein kinase RNA-activated (PKR)-like ER kinase; ATF, activating transcription factor; eIF2, eukaryotic translation initiation factor 2; BiP, binding immunoglobulin protein; Xbp1s, spliced form of X-box binding protein 1; GPCR, G protein coupled receptors; VDR, vitamin D receptor; IR, insulin resistance; ApoJ, apolipoprotein J

Key words: protein complexity, endoplasmic reticulum stress, dyslipidemia, chaperones, web-based databases, novel therapeutics

Contents

1. Introduction

Dyslipidemia is a condition of elevated cholesterol levels in the blood, which leads to premature atherosclerotic cardiovascular disease. The common clinical features of this condition are the development of xanthomas, early coronary atherosclerosis, hemolytic anemia and liver dysfunction (1). The pathogenesis of dyslipidemia involves lipid metabolism and several other signaling pathways. Some of the known risk factors include obesity and diabetes. The development and evolution of atherosclerotic lesions are influenced by the pathological processes of lipid deposition in the artery walls, localized inflammatory processes and endothelial dysfunction (2). Previous research has indicated that endoplasmic reticulum (ER) stress (ERS) signaling pathways are crucial in the development of dyslipidemia and associated cardiovascular diseases (CVDs). In eukaryotic cells, the ER plays a crucial role in calcium homeostasis, protein synthesis, folding and transport (3). ER homeostasis may be disrupted by several pathogenic conditions, including hyperlipidemia, oxidative stress and calcium

imbalance. ERS is then caused in the ER lumen by the presence of unfolded or misfolded proteins (4). Through various pathways, chronic ERS is connected to the development of cardiovascular disorders; inflammatory response mechanisms and apoptotic signaling pathways are also activated as a result of ERS during this disease phase. This has an impact on lipid metabolism, which results in dyslipidemia, cell dysfunction, and an alteration in the creation and stability of atherosclerotic plaques, all of which are vital factors in the emergence of CVD (5,6). Recent therapeutic research has also revealed an important function in a chaperone family known as heat shock protein (HSP)70, a cytoprotective molecular chaperone involved in protein folding and degradation. HSP70 is effective in treating and preventing type 2 diabetes mellitus (T2DM). As a result of ERS, mitochondrial dysfunction and inflammation, HSP70 leads to the emergence of insulin resistance (IR) as well (7,8). Due to the crucial role of ERS signaling pathways and their modulation of other pathogenic pathways, targeting ERS pathways may be the most effective therapeutic strategy for dyslipidemia. Furthermore, this may pave the way for a better understanding of the mechanisms involved and may aid the development of novel therapeutics for this condition. Thus, the present review discusses the role of the ERS response and the chaperone system associated with dyslipidemia.

2. Biogenesis of dyslipidemia

It is fascinating to note that several aspects of metabolic syndrome, including T2DM, hypertension, cerebrovascular illness and IR, are associated with dyslipidemia (9). As obesity is driven by pro-inflammatory adipokines and IR, vitamin D (VD) is associated with maintaining the lipid status; thus, dyslipidemia also plays a crucial role in obese individuals metabolically. Genes such as proprotein convertase subtilisin/kexin type 9 (PCSK9) and sphingosine-1-phosphate (S1P) are the key components that regulate low-density lipoprotein (LDL) and high-density lipoprotein (HDL) metabolism and function (10). PCSK9 is also used as a novel pharmaceutical target for lowering LDL cholesterol (LDL-C). It lowers the LDL-C intake from the circulation by promoting the LDL receptor (LDLR) breakdown and blocking LDLR recirculation to the cell surface (11). By contrast, S1P obtained by the phosphorylation of sphingosine is catalyzed by sphingosine kinase (SPHK)1 at the plasma membrane and SPHK2 in the ER, mitochondria and nucleus. S1P contributes to dyslipidemia by its concentration, i.e., a low S1P concentration leads to the vasodilation of isolated arterioles, and a high S1P concentration promotes the circulating lipids to accumulate inside the arterioles, leading to vasoconstriction (12).

Nuclear receptors, such as the peroxisome proliferator-activated receptor (PPAR) and the liver X receptor (LXR) are considered to be the transcriptional regulators in lipid metabolism, and they also play a crucial role in maintaining glucose homeostasis and inflammation by making the receptor signaling networks novel molecular targets for treating lipid-related illnesses (13). The nuclear hormone receptor superfamily's PPAR-α is a ligand-activated transcription factor that is agonistic toward fibrates. Fibrate medications are a significant class of medications used in the treatment of dyslipidemia (14). LXR, on the other hand, helps in maintaining cholesterol homeostasis by preventing the uptake of cholesterol through the intestinal tract, promoting the efflux of cholesterol from cells into HDLs, facilitating the liver's conversion of HDL into bile acids, and facilitating biliary excretion. Synthetic LXR agonists, such as T0901317 and GW3965 mainly target dyslipidemia due to their notable impact on reverse cholesterol transport, cholesterol absorption and plasma HDL (15). A diagrammatic representation of the mechanisms and functions of PPAR, SP1, LXR and PCSK9 is presented in Fig. 1.

The most frequent cause of chronic liver disease, non-alcoholic fatty liver disease (NAFLD), is considered to be the hepatic manifestation of the metabolic disease and is intricately linked to obesity, IR and dyslipidemia. Via hepatic and extrahepatic routes, insulin affects the metabolism of glucose and lipids in the liver, and IR serves as a critical factor in the emergence of dyslipidemia associated with NAFLD, which ultimately increases the risk of developing early cardiovascular illnesses, the main cause of morbidity and mortality in patients with NAFLD (16).

Several lipid molecules, such as phosphatidylcholine (PC), lysophosphatidylcholine (LPC), sphingomyelin (SM), diglyceride (DG), monoglyceride (MG) and sphingosine, are involved in alterations in individuals with dyslipidemia; in these individuals, it has been found that LPC, DG, MG and sphingosine levels are upregulated, whereas PC and SM levels are downregulated. By utilizing the phosphocholine from CDP-choline, DGs can be transformed into PCs, and CDP-choline also contributes phosphocholine for the biosynthesis of SMs. DGs interact with acyl CoA to generate triglycerides (TGs) if they are not transformed into PCs (17). A downregulation in the levels of PCs and SMs may also be related to decreased activities of triggering receptor expressed on myeloid cells 2 (TREM2), which are significant and potent ligands for activating TREM2 signals that promote macrophage activation. Thus, it could be concluded that the onset of elevated cholesterol levels in bodily fluids provokes the immune response associated with various signaling pathways involved in metabolism.

3. Proteins associated with dyslipidemia

Dyslipidemia is a medical condition that affects the levels of fat in the blood. It is characterized by increased levels of LDL-C and TGs and/or decreased levels of HDL-cholesterol. This dysfunction can lead to a high risk of developing metabolic syndrome, obesity, NAFLD or combined hyperlipidemia (18). The levels of cholesterol and TGs in the blood are closely linked to the number of LDL particles in the circulation. LDL particles bind to receptors on the surface of liver cells, including LDL receptor-related protein 1, fatty acid translocase scavenger receptor (CD36) and LDLR. LDLR is the most important of these receptors and plays a crucial role in the removal of LDL particles from the blood (19). The activity of LDLR is modulated by several factors, including hormones, growth factors and sterol regulatory element-binding protein 2 (SREBP2). SREBP2 is a transcription factor that controls the expression of the LDLR gene (20). The degradation and recirculation of LDLR to the plasma membrane surface are influenced by proprotein convertase subtilisin/kexin type 9

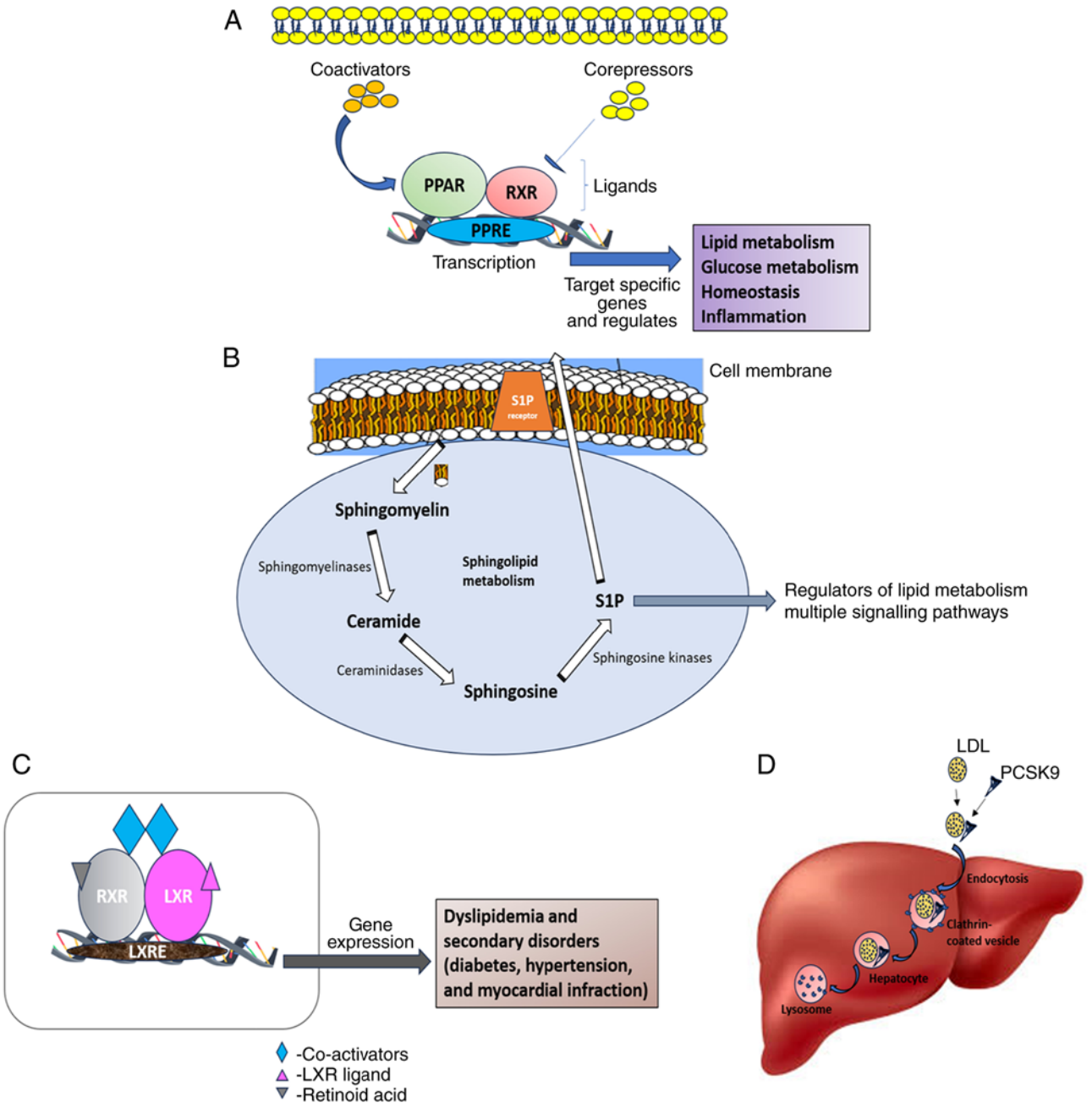

Figure 1. Schematic representation of the mechanisms and role of (A) PPAR, (B) S1P, (C) LXR, and (D) PCSK9 in dyslipidemia. PPAR, peroxisome proliferator-activated receptor; S1P, sphingosine-1-phosphate; LXR, liver X receptor; PCSK9, proprotein convertase subtilisin/kexin type 9; RXR, retinoid X receptor; PPRE, PPAR response element; LXRE, LXR response element.

serine protease (PCSK9). PCSK9 binds to the EGF-A domain of LDLR through its catalytic domain, and it directs LDLR to the endocytic pathway towards the lysosomes (21).

The interaction between PCSK9 and LDLR is another key perspective for the regulation of blood cholesterol levels. Genetic studies have demonstrated that individuals with loss-of-function mutations in PCSK9 have low cholesterol levels (22). By contrast, individuals with gain-of-function mutations in PCSK9 have high cholesterol levels. Earlier studies on PCSK9 in patients treated with various therapeutic strategies demonstrated that it may be a promising therapeutic target for dyslipidemia. In addition to LDLR, PCSK9 also enhances the degradation of CD36 (23). CD36 is a protein that has a number of lipid-related functions in the liver, such as binding free long-chain fatty acids, or LDL, facilitating their transport into the cells. The level of CD36 has also been found to be increased in hepatocytes under lipotoxic conditions or in NAFLD (24).

The PCSK9-mediated control of LDLR recycling is also influenced by another protein known as Annexin A2 (ANXA2). When ANXA2 binds to PCSK9, it causes a conformational change in the protein and hampers its binding to LDLR. This reduces the degradation of LDLR and increases its recycling to the plasma membrane surface. As previously demonstrated, LDLR levels were decreased by 20% in the liver of ANXA2-deficient mice. The reduction was even more

Table I. Effects of ceramides and lipids under different metabolic conditions.

Metabolic conditions	Effect of ceramides	Effect of lipids
Insulin sensitivity	Decreased	Decreased
Metabolic derangement	Increased	Increased
Cell death	Stimulated	Stimulated
Oxidation of LDL particles	Increased	Increased
Oxidative stress	Increased	Increased
Inflammation	Increased	Increased

marked in tissues like the adrenal gland and colon, which are known to be rich in ANXA2 and resistant to the PCSK9 effect (25,26).

Ceramides are a type of lipid that can modulate insulin sensitivity, cause metabolic derangement and stimulate cell death. They are also involved in the oxidation of LDL particles. The lipid composition of the diet can affect the levels of ceramides in the body, and different diet patterns will have differential effects on lipid profiles in hepatocytes. Some lipids are more toxic, and they induce oxidative and inflammatory processes. This can promote the progression and severity of dyslipidemia-associated diseases, such as NAFLD (27). The difference between the effects of ceramides and lipids is summarized in Table I.

The most common cause of secondary hyperlipidemia in children and adults is nephrotic syndrome, which causes the kidneys to leak protein into the urine, which leads to various health disorders, including high cholesterol levels. In patients with nephrotic syndrome, the levels of lipoproteins, including intermediate-density lipoprotein, very high LDL and LDL are elevated. This is due to the fact that the liver produces more lipoproteins in an attempt to compensate for the protein loss in the urine. In addition, patients with nephrotic syndrome exhibit a downregulation in hepatic lipase and lipoprotein lipase activities that are responsible for breaking down lipoproteins. The downregulation of such enzymatic events leads to an increase in the levels of lipoproteins in the blood. Patients with nephrotic syndrome also have high plasma PCSK9 levels (28). PCSK9 breaks down the LDL receptor to remove LDL from the blood. Thus, the high levels of PCSK9 lead to decreased LDL clearance and elevated plasma LDL-C levels. As a result of these changes, patients with nephrotic syndrome are more likely to develop atherosclerosis, which is a narrowing of the arteries that can lead to heart attack, stroke, and other cardiovascular complications. Furthermore, they are also more likely to experience progressive kidney disease and premature death (29).

4. Endoplasmic reticulum stress

In order to ensure that only proteins that have been correctly folded may reach their destination, the main role of the ER is to perform effective quality control (30). Specific chaperones of the reticular compartment tightly control this maturation process, and three groups may be formed from these: i) glucose regulated protein (GRP)78 and GRP94, which promote the folding and assembly of quasi-proteins (31); ii) lectins, calreticulin and calnexin play a crucial role in the maturation phase of glycoproteins (32), whereas in collagens; iii) HSP47 plays a major role (33). Moreover, there is a diverse functional family of enzymes, such as protein disulfide isomerases, that catalyze the synthesis or destruction of disulfide bonds [protein disulfide isomerases (PDIs)], which are essential for protein complexity. The steps involved in protein folding are illustrated in Fig. 2.

Endoplasmic reticulum-associated protein degradation (ERAD) is the process by which broken or improperly folded proteins are trapped and removed under physiological circumstances (34). Calcium is essential to this process as reticular chaperones have a range of affinities for it, and their activity is regulated by variations in its concentration (35). Folding and refolding constitute a net energy consumption in the form of ATP in any circumstance that alters intracellular concentration levels, which can inhibit their function. Reticular homeostasis is modified when the capacity of the reticular antioxidant systems is saturated as PDI activity involves a net generation of reactive oxygen species. This stress response has the power to impair the ability of protein complexes to ensure the proper folding of newly synthesized proteins, leading to aggregation and protein misfolding, a state more appropriately known as 'reticular stress' (36). Furthermore, it has been found that the unfolded protein response (UPR) signal transduction system is triggered in response to ERS. The UPR activation enables the cell to manage the increased demand for protein folding in the ER.

Moreover, apart from the biological mechanisms, no evidence has been found in clinical analyses (via biological fluids) to prove that ERS is associated with chaperones. By contrast, an important ERS moderator known as GRP78 has been studied (37). Such circumstances involve the ER-stress-dependent dysregulation of lipid metabolism and its homeostasis.

5. Role of the ER in protein complexity

In ER, misfolded proteins trigger the production of ER-resident chaperones, thus activating GRP78/binding immunoglobulin protein (BiP) as a result, and temporarily reducing protein synthesis. The UPR is regarded as an adaptive and cytoprotective mechanism as it restores ER capacity by rebalancing protein load and folding (38). The inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6) and protein kinase RNA-activated (PKR)-like ER kinase (PERK) are the three main transmembrane protein/stress sensors that regulate the UPR. These stress sensors are kept in an inactive state by the ER chaperone GRP78/BiP, which is linked to them. Unfolded or misfolded proteins bind to BiP when ERS causes it to accumulate in the ER lumen, facilitating BiP dissociation and activating downstream signaling (39,40). Homodimerization and trans-autophosphorylation are used to activate PERK when proteins are detached from Bip. The activated PERK phosphorylates the α-subunit of eukaryotic translation initiation factor 2 (eIF2) at Ser51, resulting in temporary inhibition of protein synthesis. Activating transcription factor (ATF)4 is a transcription factor whose translation is paradoxically made more efficient by eIF2 phosphorylation in post-translational

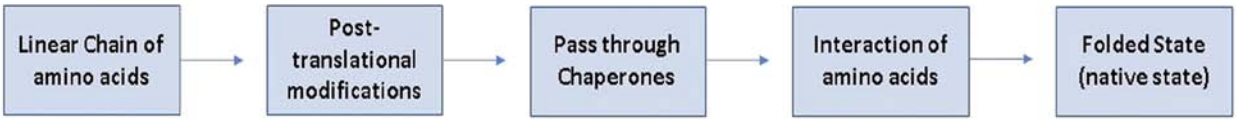

Figure 2. Steps involved in protein folding. The linear chain of amino acids in the protein undergoes a series of reactions to become a properly folded state.

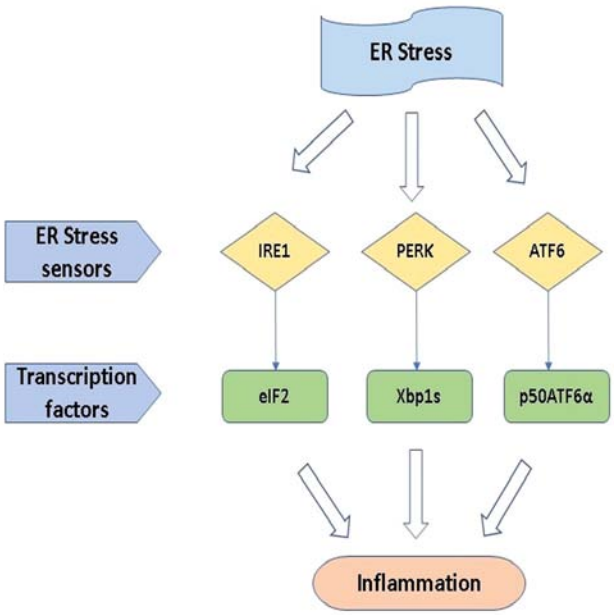

Figure 3. ER stress response in intracellular signaling. The ER stress response contribution to inflammation is schematically represented using ER stress sensors and transcription factors, respectively. ER, endoplasmic reticulum; IRE1, inositol requiring enzyme 1; PERK, protein kinase RNA-activated (PKR)-like ER kinase; ATF6, activating transcription factor 6; eIF2, eukaryotic translation initiation factor 2; Xbp1s, spliced form of X-box binding protein 1.

modifications. To dephosphorylate eIF2 and restore overall mRNA translation, ATF4 enhances the expression of its target genes, including DNA damage-inducible protein 34 and growth arrest.

IRE1 is the same as PERK; activated IRE1 produces a transcription factor (Xbp1s), and it regulates the gene expression of ER protein folding, ERAD, and protein secretion from the cell. ATF6 produces a cytosolic fragment (p50ATF6α), which acts as a transcription factor to enhance the activity of ER capacity (41). Hence, it is suggested that further *in vivo* studies tuning the transcription factors of Xbp1s and ATF6 with modified proteins or chemical components are required to overcome the stress responses caused by liver disorders and lipid abnormalities (42). The overall possibilities of the ERS response in intracellular signaling are illustrated in Fig. 3.

6. Role of chaperones in dyslipidemia

Chaperones are the molecules of the ER that encounter proteins in folding mechanisms. Generally, chaperones can be classified into three types based on their biological, chemical and pharmacological properties. Unspecific molecular chaperones are found in all living organisms and assist in protein folding. Similarly, chemical chaperones are extracellular components that aid in protein folding. By contrast, pharmacological chaperones, which are G protein coupled receptor (GPCR) ligands, are particularly beneficial for GPCR folding (43). Some of the key chaperone systems linked to dyslipidemia include the following:

i) HSP70 and HSP72. HSPs are cytoprotective as they protect proteins, lipids and nucleic acids from oxidative stress, and additionally, they influence gene expression and cell function, which contribute to maintaining protein homeostasis. Fat deposition, the accumulation of oxidants, mitochondrial malfunction and an overactive renin-angiotensin system are linked to nitric oxide (NO), and nicotinamide adenine dinucleotide phosphate oxidase activity is all associated with IR, leading to dyslipidemia. The reduced NO release in the blood promotes HSP70, which protects against oxidative stress damage, inflammation, and apoptosis in IR and diabetes, whereas HSP72 is connected to vascular complications brought on by a high-fat diet (44,45). Thus, elucidating the functions of NO and HSPs is critical for use in novel therapies, such as thermal therapy, nitrosylated medications, chemical chaperones and exercise training.

VD and vitamin D receptor (VDR) play a crucial role in the cardiovascular system and IR metabolism. Maintaining VD concentrations is critical. Koroshi and Idrizi (46) proved that HSP70 is involved in regulating the VDR concentrations of cells, and intracellular VD-binding proteins associated with HSP70 control the metabolism of VD. Thus, HSP70 in VD is concerned with the therapeutic advantage of controlling the protein expression in the cell corresponding to cardiovascular illness (47). It is fascinating to note that T2DM, hypertension, cerebrovascular illness and IR are several conditions of metabolic syndrome related to Alzheimer's disease (AD), apart from dyslipidemia.

ii) Clusterin. Clusterin, otherwise known as apolipoprotein J (ApoJ), functions as a molecular chaperone in secreted protein folding, and it has been shown that altered clusterin expression and protein levels have been linked to IR, dyslipidemia and AD associated with CVDs (48). In our view, ApoJ is a heterodimeric glycoprotein involved in muscle and nerve actions. Hence, it can be utilized in the transportation of lipid molecules from one region to another. In addition, it plays a major role in tissues and membranes in maintaining protein folding stability. Recently, ApoJ has been investigated in neurodegenerative disorders, such as Parkinson's disease (49) and AD (50).

iii) HSP47. HSP47 is a responsible chaperone in collagen folding, otherwise known as collagen-specific stress protein. *In vivo* studies on rats with hypercholesteremia supplemented with a 2% cholesterol diet have demonstrated that HSP47

Table II. Role and differences between chaperone systems.

Chaperone system	Role in dyslipidemia	Differences	Authors/(Refs.)
HSP70	Aids in the preservation of cholesterol homeostasis by encouraging the breakdown of excess cholesterol and avoiding its cellular build-up	All cells have the ubiquitous chaperone system HSP70. It is engaged in many different biological functions, such as the folding, transport, and destruction of proteins	Gungor et *al* (53), Wang *et al* (54)
HSP72	In dyslipidemia, it plays a similar role to HSP70, although it is more selectively expressed in the liver and other organs involved in lipid metabolism	Although HSP72 is a chaperone belonging to the HSP70 family, it possesses several distinct characteristics. For instance, it has a greater affinity for certain proteins and is more resistant to denaturation than HSP70	Dong *et al* (55), Johnson *et al* (56)
Clusterin	A versatile protein that chaperones lipids and lipoproteins among other types of proteins. It is considered that clusterin contributes to the prevention of cholesterol and other lipids from clumping together in dyslipidemia	Clusterin is a distinct chaperone system because it is not a member of the HSP family of chaperones. It is also the only known chaperone mechanism that is secreted from cells	Wittwer and Bradley (57), Zhu *et al* (58)
HSP47	A chaperone that plays a specific role in collagen transport across the endoplasmic reticulum membrane. HSP47 is hypothesized to have a unction in limiting cholesterol buildup in the liver in dyslipidemia	HSP47 belongs to the chaperone HSP40 family. HSP40 and HSP70 chaperones collaborate to aid in protein folding and transport	Xu *et al* (59), Sepulveda *et al* (60)

induction serves as a treatment for hypercholesteremia by contributing to glomerulosclerosis and collagen synthesis. Of note, HSP47 is abundantly involved in liver fibrosis (a condition of excess accumulation of collagen) (51) and further studies are required to shed further light on its actions.

It is known that chaperones work based on ER signals (intracellular signals), which play a significant role in post-translational modifications. Thus, the further development of chemically synthesized chaperones that have the capability to reduce ERS may serve as novel therapeutics for diseases. In addition, it has been shown that tauroursodeoxycholic acid (TUDCA; a chemical chaperone) reduces ERS and rescues hypercholesteremia in neutrophil extracellular traps (52).

A summary of the roles and differences between chaperone systems in dyslipidemia is presented in Table II (53-60).

7. Summary and conclusions

The long-term consequences of ERS on protein complexity were not discussed in the present review. To fully comprehend the long-term effects of ERS on protein folding and function, further investigations are required. The present study did not account for individual differences in ERS responsiveness. It is possible that some individuals are more vulnerable to the effects of ERS than others. Although the ERS response is implicated in other illnesses, including diabetes, obesity and cancer, the present review concentrates on the involvement of the ERS response in dyslipidemia and is based on a small amount of research. Therefore, in order to create novel therapeutics that target the ERS response, further studies are required to confirm the findings discussed herein and clarify the function of protein complexity and the ERS response in dyslipidemia. Furthermore, to comprehend the significance of the ERS response in other disorders with the potential to significantly influence the development of novel therapeutics for dyslipidemia, the current review offers a fresh and thorough assessment of the function of protein complexity and the ERS response in dyslipidemia.

The present review provides an overview of the roles played by protein complexity and the ERS response in dyslipidemia. A significant risk factor for CVD is characterized by high blood levels of TGs and/or cholesterol. Due to oxidative stress, calcium imbalance and elevated cholesterol levels, dyslipidemia can result in an overflow of unfolded proteins in the ER. This sets off the ERS response, which can cause inflammation and mortality; however, it can also have an adaptive effect by upregulating chaperone expression and aiding in the restoration of ER function.

Aside from highlighting the significance of taking oxidative stress, calcium imbalance and high cholesterol into account when developing novel therapeutics for dyslipidemia, the present review also provides new insight into the role of

protein complexity and the ERS response in the disease. It also discusses the role of chaperones in the ERS response and how this may be used to develop novel therapies for dyslipidemia.

8. Suggestions and future perspectives

The identification of multiple roles for ER in physiology and disease was made possible by the progression in our understanding of ER activities. Relevant opportunities to make use of this knowledge were made possible by the ER, and several aspects were identified, including homeostatic regulators and the UPR. The UPR connects with a variety of physiological functions, including protein folding, transcriptional regulation, translational control, protein degradation, and the regulation of signaling pathways that determine the destiny of a cell. Moreover, ERS regulators and chaperones are considered to be novel molecular diagnostic indicators and therapeutic targets. With this salient point, some of the key techniques and molecules for the development of future novel therapeutics are provide below, which include clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9), TUDCA, adenosine monophosphate activated protein kinase (AMPK), C/EBP homologous protein (CHOP) and PH domain and leucine rich repeat protein phosphatase 1 (PHLPP1).

i) FDA-approved compounds that can be utilized in human medicine include 4-phenylbutyric acid and TUDCA, which are chemically synthesized chaperones. It has been proven that TUDCA can relieve ERS and prevent hypercholesterolemia in macrophages (52). Additionally, sirtuin-1 chaperone activity is regulated to control raptor acetylation, which in turn suppresses mammalian target of rapamycin complex 1-dependent protein synthesis and decreases ER overload (61).

ii) CHOP drives the main proapoptotic pathways brought on by ERS. Hence, CHOP inhibitors can be designed selectively to target the host to achieve a reduced ERS response. Research on CHOP activation has determined that the upregulation of microRNAs (miR-33) in atherosclerotic macrophages disrupted the ERS response in lipid metabolism (62).

iii) PHLPP1, when disrupted using CRISPR/Cas9, zebrafish larvae and *C. elegans*, exhibits less lipid deposition in their intersegmental arteries, as well as lower levels of total cholesterol and TGs *in vivo* analysis (63). Thus, PHLPP1 can be utilized in similar animal models to reduce the accumulation of lipids in the arteries.

iv) The AMPK signaling pathway is considered to regulate ERS (64). AMPK functions as a physiological ERS inhibitor, and the effect of inhibition is maintained by the sarco-endoplasmic reticulum calcium ATPase activity and intracellular Ca^{2+}. Among the widely used statin drug types in dyslipidemia, it has been shown that atorvastatin is an extensively researched pharmaceutical drug that can decrease ERS through activating AMPK in *in vivo* studies on atherosclerotic mice and cultured human umbilical vein-derived endothelial cells (65).

v) Additionally, it is suggested that utilizing herbal remedies may also serve as a preventative against dyslipidemia by inhibiting the signaling pathways that are upregulated during ERS. For instance, a study in the journal 'Phytomedicine', found that the herb ginger (*Zingiber officinale*) was able to inhibit the expression of genes involved in ERS and inflammation in mice with high cholesterol levels. The study also found that ginger was able to reduce the levels of cholesterol and TGs in the blood of mice (66). Another study was conducted on turmeric (*Curcuma longa*) and explored similar results to ginger (67). The mechanisms by which ginger and turmeric exert these effects are not yet fully understood, although it is considered that they may involve the inhibition of enzymes called kinases, which are involved in the signaling pathways that are upregulated during ERS. The detailed perspectives and mechanisms involved are under research that may conclude with an alternate remedy to decrease the levels of cholesterol and TGs in the blood (66,68).

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

MSAH was involved in the conceptualization of the study, in the submission of the final manuscript and in correspondence. RK was involved in the reviewing and editing of the manuscript. MSAH and RK were involved in the literature search for studies to be included in the review. All the authors have read and approved the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. García-Giustiniani D and Stein R: Genetics of Dyslipidemia. Arq Bras Cardiol 106: 434-438, 2016.
2. Fuster JJ: Integrated stress response inhibition in atherosclerosis. Preventing the Stressed-Out Plaque. J Am Coll Cardiol 73: 1170-1172, 2019.
3. Engin F and Hotamisligil GS: Restoring endoplasmic reticulum function by chemical chaperones: An emerging therapeutic approach for metabolic diseases. Diabetes Obes Metab 12 (Suppl 2): S108-S115, 2010.

4. Dandekar A, Mendez R and Zhang K: Cross talk between er stress, oxidative stress, and inflammation in health and disease. Methods Mol Biol 1292: 205-214, 2015.
5. Kruzliak P, Sabo J and Zulli A: Endothelial endoplasmic reticulum and nitrative stress in endothelial dysfunction in the atherogenic rabbit model. Acta Histochemica 117: 762-766, 2015.
6. Zhang C, Syed TW, Liu R and Yu J: Role of endoplasmic reticulum stress, autophagy, and inflammation in cardiovascular disease. Front Cardiovasc Med 4: 29, 2017.
7. Esser N, Legrand-Poels S, Piette J, Scheen AJ and Paquot N: Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res Clin Pract 105: 141-150, 2014.
8. Krause M, Bock PM, Takahashi HK, Homem De Bittencourt PI Jr and Newsholme P: The regulatory roles of NADPH oxidase, intra- and extra-cellular HSP70 in pancreatic islet function, dysfunction and diabetes. Clin Sci (Lond) 128: 789-803, 2015.
9. Gottesman RF, Albert MS, Alonso A, Coker LH, Coresh J, Davis SM, Deal JA, McKhann GM, Mosley TH, Sharrett AR, *et al*: Associations Between Midlife Vascular Risk Factors and 25-Year Incident Dementia in the Atherosclerosis Risk in Communities (ARIC) Cohort. JAMA Neurol 74: 1246-1254, 2017.
10. Vekic J, Zeljkovic A, Stefanovic A, Jelic-Ivanovic Z and Spasojevic-Kalimanovska V: Obesity and dyslipidemia. Metabolism 92: 71-81, 2019.
11. Lin XL, Xiao LL, Tang ZH, Jiang ZS and Liu MH: Role of PCSK9 in lipid metabolism and atherosclerosis. Biomed Pharmacother 104: 36-44, 2018.
12. Chen W, Lu H, Yang J, Xiang H and Peng H: Sphingosine 1-phosphate in metabolic syndrome (Review). Int J Mol Med 38: 1030-1038, 2016.
13. Beaven SW and Tontonoz P: Nuclear receptors in lipid metabolism: Targeting the heart of dyslipidemia. Annu Rev Med 57: 313-329, 2006.
14. Botta M, Audano M, Sahebkar A, Sirtori CR, Mitro N and Ruscica M: PPAR Agonists and Metabolic Syndrome: An Established Role? Int J Mol Sci 19: 1197, 2018.
15. Wang B and Tontonoz P: Liver X receptors in lipid signalling and membrane homeostasis. Nat Rev Endocrinol 14: 452-463, 2018.
16. Akhtar DH, Iqbal U, Vazquez-Montesino LM, Dennis BB and Ahmed A: Pathogenesis of insulin resistance and atherogenic dyslipidemia in nonalcoholic fatty liver disease. J Clin Transl Hepatol 7: 362-370, 2019.
17. Ke C, Zhu X, Zhang Y and Shen Y: Metabolomic characterization of hypertension and dyslipidemia. Metabolomics 14: 117, 2018.
18. Csonka C, Baranyai T, Tiszlavicz L, Fébel H, Szűcs G, Varga ZV, Sárközy M, Puskás LG, Antal O, Siska A, *et al*: Isolated hypercholesterolemia leads to steatosis in the liver without affecting the pancreas. Lipids Health Dis 16: 144, 2017.
19. Begg MJ, Sturrock ED and van der Westhuyzen DR: Soluble LDL-R are formed by cell surface cleavage in response to phorbol esters. Eur J Biochem 271: 524-533, 2004.
20. Streicher R, Kotzka J, Müller-Wieland D, Siemeister G, Munck M, Avci H and Krone W: SREBP-1 Mediates Activation of the Low Density Lipoprotein Receptor Promoter by Insulin and Insulin-like Growth Factor-I. J Biol Chem 271: 7128-7133, 1996.
21. Kwon HJ, Lagace TA, McNutt MC, Horton JD and Deisenhofer J: Molecular basis for LDL receptor recognition by PCSK9. Proc Natl Acad Sci USA 105: 1820-1825, 2008.
22. Demers A, Samami S, Lauzier B, Des Rosiers C, Ngo Sock ET, Ong H and Mayer G: PCSK9 Induces CD36 Degradation and Affects Long-Chain Fatty Acid Uptake and Triglyceride Metabolism in Adipocytes and in Mouse Liver. Arterioscler Thromb Vasc Biol 35: 2517-2525, 2015.
23. Pepino MY, Kuda O, Samovski D and Abumrad NA: Structure-Function of CD36 and Importance of Fatty Acid Signal Transduction in Fat Metabolism. Annu Rev Nutr 34: 281-303, 2014.
24. Greco D, Kotronen A, Westerbacka J, Puig O, Arkkila P, Kiviluoto T, Laitinen S, Kolak M, Fisher RM, Hamsten A, *et al*: Gene expression in human NAFLD. Am J Physiol Gastrointest Liver Physiol 294: G1281-G1287, 2008.
25. Zaid A, Roubtsova A, Essalmani R, Marcinkiewicz J, Chamberland A, Hamelin J, Tremblay M, Jacques H, Jin W, Davignon J, *et al*: Proprotein convertase subtilisin/kexin type 9 (PCSK9): Hepatocyte-specific low-density lipoprotein receptor degradation and critical role in mouse liver regeneration. Hepatology 48: 646-654, 2008.
26. Seidah NG, Poirier S, Denis M, Parker R, Miao B, Mapelli C, Prat A, Wassef H, Davignon J, Hajjar KA and Mayer G: Annexin A2 is a natural extrahepatic inhibitor of the PCSK9-Induced LDL receptor degradation. PLoS One 7: e41865, 2012.
27. Németh K, Tóth B, Sarnyai F, Koncz A, Lenzinger D, Kereszturi É, Visnovitz T, Kestecher BM, Osteikoetxea X, Csala M, *et al*: High fat diet and PCSK9 knockout modulates lipid profile of the liver and changes the expression of lipid homeostasis related genes. Nutr Metab (Lond) 20: 19, 2023.
28. Agrawal S, Zaritsky JJ, Fornoni A and Smoyer WE: Dyslipidaemia in nephrotic syndrome: Mechanisms and treatment. Nat Rev Nephrol 14: 57-70, 2018.
29. Kongmalai T, Chuanchaiyakul N, Srinoulprasert Y and Thongtang N: Injection of an improperly stored proprotein convertase subtilisin/kexin type 9 monoclonal antibody in a patient with secondary dyslipidemia from nephrotic syndrome: A case report. J Med Case Rep 17: 89, 2023.
30. Almanza A, Carlesso A, Chintha C, Creedican S, Doultsinos D, Leuzzi B, Luís A, McCarthy N, Montibeller L, More S, *et al*: Endoplasmic reticulum stress signalling – from basic mechanisms to clinical applications. FEBS J 286: 241-278, 2019.
31. Fu XL and Gao DS: Endoplasmic reticulum proteins quality control and the unfolded protein response: The regulative mechanism of organisms against stress injuries. BioFactors 40: 569-585, 2014.
32. Williams DB: Beyond lectins: The calnexin/calreticulin chaperone system of the endoplasmic reticulum. J Cell Sci 119(Pt 4): 615-623, 2006.
33. Ishida Y and Nagata K: Hsp47 as a collagen-specific molecular chaperone. Methods Enzymol 499: 167-182, 2011.
34. Hirsch C, Gauss R, Horn SC, Neuber O and Sommer T: The ubiquitylation machinery of the endoplasmic reticulum. Nature 458: 453-460, 2009.
35. Coe H and Michalak M: Calcium binding chaperones of the endoplasmic reticulum. Gen Physiol Biophys 28 Spec No Focus: F96-F103, 2009.
36. Bhandary B, Marahatta A, Kim HR and Chae HJ: An involvement of oxidative stress in endoplasmic reticulum stress and its associated diseases. Int J Mol Sci 14: 434-456, 2012.
37. Al Zaidi M, Repges E, Sommer-Weisel S, Jansen F, Zimmer S, Tiyerili V, Nickenig G and Aksoy A: Serum levels of the endoplasmic-reticulum-stress chaperone GRP78 identify patients with coronary artery disease and predict mortality. Eur Heart J 43 Suppl2: ehac544.1137, 2022.
38. Sannino S and Brodsky JL: Targeting protein quality control pathways in breast cancer. BMC Biol 15: 109, 2017.
39. Bertolotti A, Zhang Y, Hendershot LM, Harding HP and Ron D: Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol 2: 326-332, 2000.
40. Shen J, Chen X, Hendershot L and Prywes R: ER Stress Regulation of ATF6 Localization by Dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. Dev Cell 3: 99-111, 2002.
41. Han J and Kaufman RJ: The role of ER stress in lipid metabolism and lipotoxicity. J Lipid Res 57: 1329-1338, 2016.
42. Liu X, Khalafalla M, Chung C, Gindin Y, Hubchak S, LeCuyer B, Kriegermeier A, Zhang D, Qiu W, Ding X, *et al*: Hepatic Deletion of X-Box Binding Protein 1 in FXR Null mice leads to enhanced liver injury. J Lipid Res 63: 100289, 2022.
43. Tao YX and Conn PM: Chaperoning G Protein-Coupled Receptors: From cell biology to therapeutics. Endocr Rev 35: 602-647, 2014.
44. Macario AJ and Conway de Macario E: Sick chaperones, cellular stress, and disease. N Engl J Med 353: 1489-1501, 2005.
45. Molina MN, Ferder L and Manucha W: Emerging role of nitric oxide and heat shock proteins in insulin resistance. Curr Hypertens Rep 18: 1, 2015.
46. Koroshi A and Idrizi A: Renoprotective effects of Vitamin D and renin-angiotensin system. Hippokratia 15: 308-311, 2011.
47. Adams JS, Chen H, Chun RF, Nguyen L, Wu S, Ren SY, Barsony J and Gacad MA: Novel regulators of vitamin D action and metabolism: Lessons learned at the Los Angeles zoo. J Cell Biochem 88: 308-314, 2003.
48. Bradley D: Clusterin as a potential biomarker of obesity-related Alzheimer's disease risk. Biomark Insights 15: 1177271920964108, 2020.
49. Berdowska I, Matusiewicz M and Krzystek-Korpacka M: HDL Accessory proteins in parkinson's disease-focusing on clusterin (Apolipoprotein J) in regard to its involvement in pathology and diagnostics-A review. Antioxidants (Basel) 11: 524, 2022.

50. Fareed MM, Qasmi M, Aziz S, Völker E, Förster CY and Shityakov S: The role of clusterin transporter in the pathogenesis of Alzheimer's disease at the blood-brain barrier interface: A systematic review. Biomolecules 12: 1452, 2022.
51. Yuan W, Qiu T, Yao X, Wu C, Shi Y, Wang N, Zhang J, Jiang L, Liu X, Yang G, *et al*: Hsp47 acts as a bridge between NLRP3 inflammasome and hepatic stellate cells activation in arsenic-induced liver fibrosis. Toxicol Lett 370: 7-14, 2022.
52. Dhawan UK, Bhattacharya P, Narayanan S, Manickam V, Aggarwal A and Subramanian M: Hypercholesterolemia Impairs Clearance of Neutrophil Extracellular Traps and Promotes Inflammation and Atherosclerotic Plaque Progression. Arterioscler Thromb Vasc Biol 41: 2598-2615, 2021.
53. Gungor B, Vanharanta L, Hölttä-Vuori M, Pirhonen J, Petersen NHT, Gramolelli S, Ojala PM, Kirkegaard T and Ikonen E: HSP70 induces liver X receptor pathway activation and cholesterol reduction in vitro and in vivo. Mol Metab 28: 135-143, 2019.
54. Wang X, Chen M, Zhou J and Zhang X: HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy. Int J Oncol 45: 18-30, 2014.
55. Dong Y, Ma N, Fan L, Yuan L, Wu Q, Gong L, Tao Z, Chen J and Ren J: GADD45β stabilized by direct interaction with HSP72 ameliorates insulin resistance and lipid accumulation. Pharmacol Res 173: 105879, 2021.
56. Johnson CN, McCoin CS, Kueck PJ, Hawley AG, John CS, Thyfault JP, Swerdlow RH, Geiger PC and Morris JK: Relationship of muscle apolipoprotein e expression with markers of cellular stress, metabolism, and blood biomarkers in cognitively healthy and impaired older adults. J Alzheimers Dis 92: 1027-1035, 2023.
57. Wittwer J and Bradley D: Clusterin and its role in insulin resistance and the cardiometabolic Syndrome. Front Immunol 12: 612496, 2021.
58. Zhu H, Liu M, Zhai T, Pan H, Wang L, Yang H, Yan K, Gong F and Zeng Y: High serum clusterin levels are associated with premature coronary artery disease in a Chinese population. Diabetes Metab Res Rev 35: e3128, 2019.
59. Xu H, Shen Y, Liang C, Wang H, Huang J, Xue P and Luo M: Inhibition of the mevalonate pathway improves myocardial fibrosis. Exp Ther Med 21: 224, 2021.
60. Sepulveda D, Rojas-Rivera D, Rodríguez DA, Groenendyk J, Köhler A, Lebeaupin C, Ito S, Urra H, Carreras-Sureda A, Hazari Y, *et al*: Interactome screening identifies the ER Luminal Chaperone Hsp47 as a regulator of the unfolded protein response transducer IRE1α. Mol Cell 69: 238-252.e7, 2018.
61. Fiorentino TV, Procopio T, Mancuso E, Arcidiacono GP, Andreozzi F, Arturi F, Sciacqua A, Perticone F, Hribal ML and Sesti G: SRT1720 counteracts glucosamine-induced endoplasmic reticulum stress and endothelial dysfunction. Cardiovasc Res 107: 295-306, 2015.
62. Sun Y, Zhang D, Liu X, Li X, Liu F, Yu Y, Jia S, Zhou Y and Zhao Y: Endoplasmic reticulum stress affects lipid metabolism in atherosclerosis via CHOP activation and over-expression of miR-33. Cell Physiol Biochem 48: 1995-2010, 2018.
63. Balamurugan K, Medishetti R, Kotha J, Behera P, Chandra K, Mavuduru VA, Joshi MB, Samineni R, Katika MR, Ball WB, *et al*: PHLPP1 promotes neutral lipid accumulation through AMPK/ChREBP-dependent lipid uptake and fatty acid synthesis pathways. iScience 25: 103766, 2022.
64. Gao F, Chen J and Zhu H: A potential strategy for treating atherosclerosis: Improving endothelial function via AMP-activated protein kinase. Sci China Life Sci 61: 1024-1029, 2018.
65. Zhao WB, Fu H, Chang F, Liu J, Wang J, Li F and Zhao J: Effects of various doses of atorvastatin on vascular endothelial cell apoptosis and autophagy in vitro. Mol Med Rep 19: 1919-1925, 2019.
66. Carnuta MG, Deleanu M, Barbalata T, Toma L, Raileanu M, Sima AV and Stancu CS: Zingiber officinale extract administration diminishes steroyl-CoA desaturase gene expression and activity in hyperlipidemic hamster liver by reducing the oxidative and endoplasmic reticulum stress. Phytomedicine 48: 62-69, 2018.
67. Zhu H, Fan Y, Sun H, Chen L and Man X: Curcumin inhibits endoplasmic reticulum stress induced by cerebral ischemia-reperfusion injury in rats. Exp Ther Med 14: 4047-4052, 2017.
68. Cai M, Wang H, Li JJ, Zhang YL, Xin L, Li F and Lou SJ: The signaling mechanisms of hippocampal endoplasmic reticulum stress affecting neuronal plasticity-related protein levels in high fat diet-induced obese rats and the regulation of aerobic exercise. Brain Behav Immun 57: 347-359, 2016.