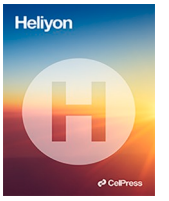
Heliyon 10 (2024) e40502

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

L-carnitine ameliorates myocardial injury by alleviating endoplasmic reticulum stress via inhibition of PERK pathway in exertional heatstroke rats

Bo-Yi Zhang [a,b,1], Gen-Lin He [a,b,1,*], Ze-Ze Wang [a,b], Huan Zhou [a,b], Xue-Yan Huang [a,b], Ting-Ting Shen [a,b], Xiao-Qian Liu [a,b], Yi-Shan Liu [a,b], Zhen Luo [a,b], Ping Li [a,b], Yu-Long Tan [a,b], Xue Luo [a,b], Xue-Sen Yang [a,b,**]

[a] *Department of Tropical Medicine, Army Medical University, Chongqing, China*
[b] *Key Laboratory of Extreme Environmental Medicine, Ministry of Education of China, Army Medical University, Chongqing, China*

ARTICLE INFO

Keywords:
L-carnitine
Exertional heatstroke
Myocardial apoptosis
Endoplasmic reticulum stress
Mitochondrial damage

ABSTRACT

Exertional heatstroke (EHS) is a life-threatening condition with potential for tissues and organs injury, including heart. Effective drug strategies to treat patients with EHS are warranted to unlock the therapeutic potential. Considering the cardioprotective effects of L-carnitine (LC), this study aimed to investigate the effects of LC on EHS-induced myocardial injury in rats and to explore the underlying mechanisms. Here, we found that LC exerted a greater protective effect on EHS-induced cardiac dysfunction and mortality, which also significantly attenuated certain negative effects, including increased myocardial apoptosis, pathological changes, and ultrastructural impairment, enhanced activity levels of such serum enzymes as AST, LDH, CK, and CK-MB, reduced BCL-2 expression, increased the expression of cleaved caspase-3 and the critical endoplasmic reticulum stress (ERS) indices like CHOP and GRP78 in EHS rats. Besides, pretreatment of EHS rats with PBA (4-Phenyl butyric acid), a chemical chaperone that attenuates ERS, restored BCL-2 expression, reduced the protein levels of cleaved caspase-3, CHOP, and GRP78. Furthermore, thapsigargin (TG), which induces ERS, enhanced the expression of BAX, cleaved caspase-3, CHOP, and GRP78, attenuated BCL-2 expression, and enhanced mitochondrial impairment in EHS + LC rats. Mechanismly, the protective effects of LC were mediated, at least partly, by inhibiting the activation of PERK pathway against ERS-associated myocardial damage. These results indicate that supplementation of LC might be a potential strategy to reduce myocardial injury by affecting ERS via inhibiting the PERK pathway against EHS.

* Corresponding author. Department of Tropical Medicine, Army Medical University, 30 Gaotanyan Central Street, Shapingba District, Chongqing, 400038, China.
** Corresponding author. Department of Tropical Medicine, Army Medical University, 30 Gaotanyan Central Street, Shapingba District, Chongqing, 400038, China.
E-mail addresses: glen0716@tmmu.edu.cn (G.-L. He), xuesenyyy@163.com (X.-S. Yang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e40502
Received 4 August 2024; Received in revised form 13 November 2024; Accepted 16 November 2024
Available online 17 November 2024

1. Introduction

Exertional heatstroke (EHS) is a life-threatening medical emergency that considered as the most serious manifestation of exertional heat injury. Global studies suggest an increasing trend of incidence of EHS during recent decades [1]. The pathophysiology of EHS is related to severe hyperthermia and follow-on tissue damage or multi-organ injury [2]. The heart, being a vulnerable organ in heat injury, functions as one of the key organ for thermoregulation, cardiovascular function, and tissue perfusion [3]. Notably, the cardinal pathologic features of EHS involves thermoregulatory and cardiovascular overload, both associated with cardiac output. Indeed, clinical evidence has revealed that EHS patients with myocardial injury had more severe clinical conditions, such as increased 90-day mortality and shortened survival time [4]. In addition, prior heatstroke was associated with an increased incidence of cardiovascular diseases [5]. It is widely accepted that beneficial strategies against EHS may be largely attributable to the cardioprotective response.

Clinical features and treatment of EHS can mimic many other illnesses including sepsis, ischemic stroke, and toxicologic emergencies, in the absence of the efficacy of targeted strategies [6]. Generally, EHS patients may present with multi-organ dysfunction that is managed conventionally [7]. Abnormalities in the pro-inflammatory and anti-inflammatory balance, cardiomyocyte death, and metabolic dysregulation are among the factors involved in heatstroke-induced myocardial injury [3]. It is therefore conceivable that the critical interventions against cardiovascular dysfunction in EHS may be multi-faceted, and require elucidation of the mechanism of EHS-induced myocardial injury. Certain studies have shown that heatstroke can induce apoptosis through different mechanisms, such as endoplasmic reticulum stress (ERS) [8], mitochondrial apoptosis pathway [9], and death receptors [10].

ERS is intricately linked to proteostasis that deal with the misfolded and unfolded proteins, and is crucial in the pathology of various heart diseases, including heat stress-induced cardiac dysfunction [11]. Current evidences emphasize the accumulation of heat-induced ERS in other tissues, such as cerebellum [12], cortex [13], and intestines [8]. Moreover, it has been proposed that ERS accompanied with mitochondrial damage, and oxidative damage following heat stress [14]. Theoretically, multi-targeted biological effects of pharmacologic compounds have become important research focus in the prevention and treatment of EHS.

L-carnitine (LC), a compound conditionally synthesized from the amino acids lysine and methionine, possesses anti-oxidative, anti-inflammatory, anti-proteolytic, and anti-apoptotic properties [15,16]. There is growing evidence that LC provide beneficial effects in various pathological conditions, including myocardial injury [17], lung injury [18], traumatic brain injury [19], skin tissue injury [20]. Noteworthy, recent studies indicate that LC supplementation might protect cardiomyocytes from oxidative stress-related damage [21,22]. Although the underlying mechanisms have not been clearly elucidated, the aforementioned data suggest that LC ability to counteract ERS [23]. To this end, the aim of our study was to investigate whether ERS involved in EHS-induced myocardial injury in rats, and to clarify the advantages and potential mechanisms of LC treatment for myocardial protection in EHS.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (8 weeks old, weighing 220–250 g) were purchased from the Experimental Animal Center of Army Medicine University, Chongqing, China. The rats were housed on a 12-h/12-h light/dark cycle at a comfortable temperature (23±1 °C) and 55±5 % relative humidity (RH) with adequate ventilation and provided food and water ad libitum unless otherwise specified. All experiments were approved by the Institutional Animal Ethics Committee of the Army Medical University with the approval number: AMUWEC2020078, and performed in accordance with the Guide for the Care and Use of Laboratory Animals – Chinese Version (1996). The 8-week-old rats were randomly divided into 8 groups (n = 10–15 per group): Con, the sham-treated control group; LC, the l-carnitine (LC)-treated group; EHS, the exertional heatstroke (EHS) group; EHS + LC, the LC-pretreated EHS group; 4-PBA, the 4-Phenyl butyric acid (4-PBA, a potent ERS inhibitor)-treated group; EHS+4-PBA, the 4-PBA-pretreated EHS group; TG, thapsigargin (TG, a known ERS activator)-treated group; and EHS + LC + TG, the TG plus LC-pretreated EHS group.

2.2. Drug administration

LC was purchased from Macklin Biochemical Technology (Shanghai, CN). 4-PBA and TG were purchased from Sigma-Aldrich (St. Louis, MO, USA). The LC group and EHS + LC group animals were treated with L-carnitine in dose of 100 mg/kg for 7 consecutive days. The 4-PBA solution was prepared by titrating equimolar amounts of 4-PBA and sodium hydroxide to pH 7.4. The 4-PBA group and EHS+4-PBA group animals were administered 4-PBA via intraperitoneal injection at a dose of 100 mg/kg/d for 7 consecutive days. The rats in the EHS model and control groups were injected with 5 mL/kg/d of 0.9 % saline for 7 consecutive days. TG was freshly mixed in a container and administered intraperitoneally as a mixture in a single injection. Rats were injected with TG (0.5 mg/kg) or vehicle alone (5 % DMSO, 40 % PEG 300, 5 % Tween 80, 50 % saline). On the eighth day of the experiment, the rats in each group were anaesthetized by an intraperitoneal injection of sodium pentobarbital (40 mg/kg).

2.3. EHS model

For familiarization with the running mode, the rats were given an adaptive treadmill training, at a speed of 12 m/min with slope 0 for 10 min/day for 5 consecutive days in 25 °C and 55 ± 3 % RH conditions, followed by two rest days to avoid impacts of training, in a large environmental simulation chamber (GFQ5500, HOTO Oxygen Industrial, Yantai, CN). In the EHS protocol used in the current study, animals were exposed to environmental temperature of 39.5 ± 0.5 °C at 55 ± 3 % RH while exercising on a forced treadmill.

Running speed was set at a constant speed of 12 m/min with slope 0. The rats were encouraged to run using light electrical stimulation (0.8 mA). The rectal temperature of rats was monitored at 8-min intervals or shorter intervals when approaching the high-temperature threshold using a thermometer (SE309, Center Technology Corp., Taiwan, CN). The study design considered rectal temperature of 42.3 °C or suffered from fatigue status as the onset of heatstroke, which mirrors many of the pathophysiological outcomes observed in human EHS, including loss of consciousness, severe hyperthermia. Then, the rat was removed from the chamber immediately for recovery in ambient temperature. At the same time, the rat in the pharmacological intervened group with a maximal temperature rise was matched in pairs and removed from the chamber.

2.4. Analysis of survival rate

The rats were randomly assigned to two experimental groups, EHS and EHS + LC. After EHS, rats were removed from the heat, and placed back into the cage with ad libitum food and water for undisturbed recovery at 25 ± 2 °C. Animals were monitored for signs of morbidity every15 min after EHS. Animals were euthanized if they displayed morbid symptoms of imminent death, including reduced locomotion, no response to tail pinch, paralysis, ataxia, and altered breath frequency. Time to death was recorded based on the humane euthanasia of LC-treated and non-treated rats after EHS. Survival data were calculated as percent survival versus time immediately after EHS.

2.5. Echocardiography

Echocardiography was conducted at the threshold 2-h recovery time after the onset of EHS (2 h after EHS onset) using a small animal high resolution ultrasound imaging system (Vevo 2100, VisualSonics, Toronto, Canada). Briefly, the rats were anaesthetized by 3 % isoflurane, and the hair on the chest was carefully removed. M-mode echocardiography indices of left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD) over the course of at least three consecutive cardiac cycles were obtained and analyzed for the left ventricular ejection fraction (LVEF) and left ventricular fraction shortening (LVFS). LVFS was calculated as (LVEDD-LVESD)/LVEDD × 100 %; left ventricular end diastolic volume (LVEDV) was calculated as $7.0 \times LVEDD^3/(2.4+LVEDD)$; left ventricular end systolic volume (LVESV) was calculated as $7.0 \times LVESD^3/(2.4+LVESD)$; LVEF was calculated as (LVEDV-LVESV)/LVEDV × 100 %. All measurements were conducted by an experienced sonographer who was blinded to the experimental design and the group assignments. Considering the similarity in rat and human echocardiography, and the heterogeneity in different studies, rats with LVEF <60 % and LVFS <35 % were considered as left ventricular dysfunction.

2.6. Serum levels of AST, LDH, CK, and CKMB detection

The rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg). The blood was collected from the abdominal aorta immediately, then serum was separated via centrifugation at 3000 rpm for 15 min at 4 °C and stored at −80 °C until use for detection. The levels of aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), and creatine kinase myocardial band (CKMB) in the serum of each group were detected according to the MNCHIP kit instructions, and analyzed on a chemistry analyzer (Pointcare M4, MNCHIP, Tianjin, CN).

2.7. Histological examination

Myocardium tissues of sacrificed rats was quickly removed from chest cavity at 2 h after EHS onset. Tissue samples were fixed in 4 % pre-cooled paraformaldehyde and embedded in paraffin. Paraffin-embedded tissues were serially sectioned into histological sections (5 μm thick). Sections were deparaffinized in xylene, and hydrated in ethanol followed by hematoxylin and eosin (H&E) staining. Images were visualized under a slide scanner (VS200, Olympus, Tokyo, Japan).

2.8. TUNEL staining

To detect apoptosis, the sections were performed as previously described [24], and then were stained with a terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) fluorescence kit (Roche Diagnostics Corp, Switzerland) according to the manufacturer's instructions. After staining, all sections were observed and photographed using a microscope (DM2000 LED, Leica, Wetzlar, Germany).

2.9. Transmission electron microscopic (TEM) examination

Freshly isolated cardiac samples were rinsed quickly in cold PBS, and then cut into smaller pieces (approximately 3 × 3 mm) to allow for optimal submersion fixation in a mixture of 2 % formaldehyde and 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 3 h at room temperature and left overnight at 4 °C. TEM examination were performed as previously described [24]. After polymerization, the ultrathin sections were counterstained with 3 % uranyl acetate and 2.7 % lead citrate. Finally, these sections were viewed using transmission electron microscopy (TEM; FEI TECNAI10, Philips Electron Optics, Eindhoven, Netherlands).

2.10. Immunoblot analysis

The samples were washed with ice-cold PBS and homogenized in RIPA lysis buffer (Beyotime, Nantong, Jiangsu, CN) containing protease and phosphatase inhibitors (Roche, Penzberg, Germany) for 30 min on ice. The lysates were centrifuged at 12,000 rpm for 10 min at 4 °C. The protein samples (80 μg/lane) were separated on 6 or 10 % SDS-polyacrylamide gels and then transferred onto PVDF membranes (BioRad, Hercules, CA, USA). The membranes were blocked in PBS with 5 % non-fat milk for 1 h and then incubated with

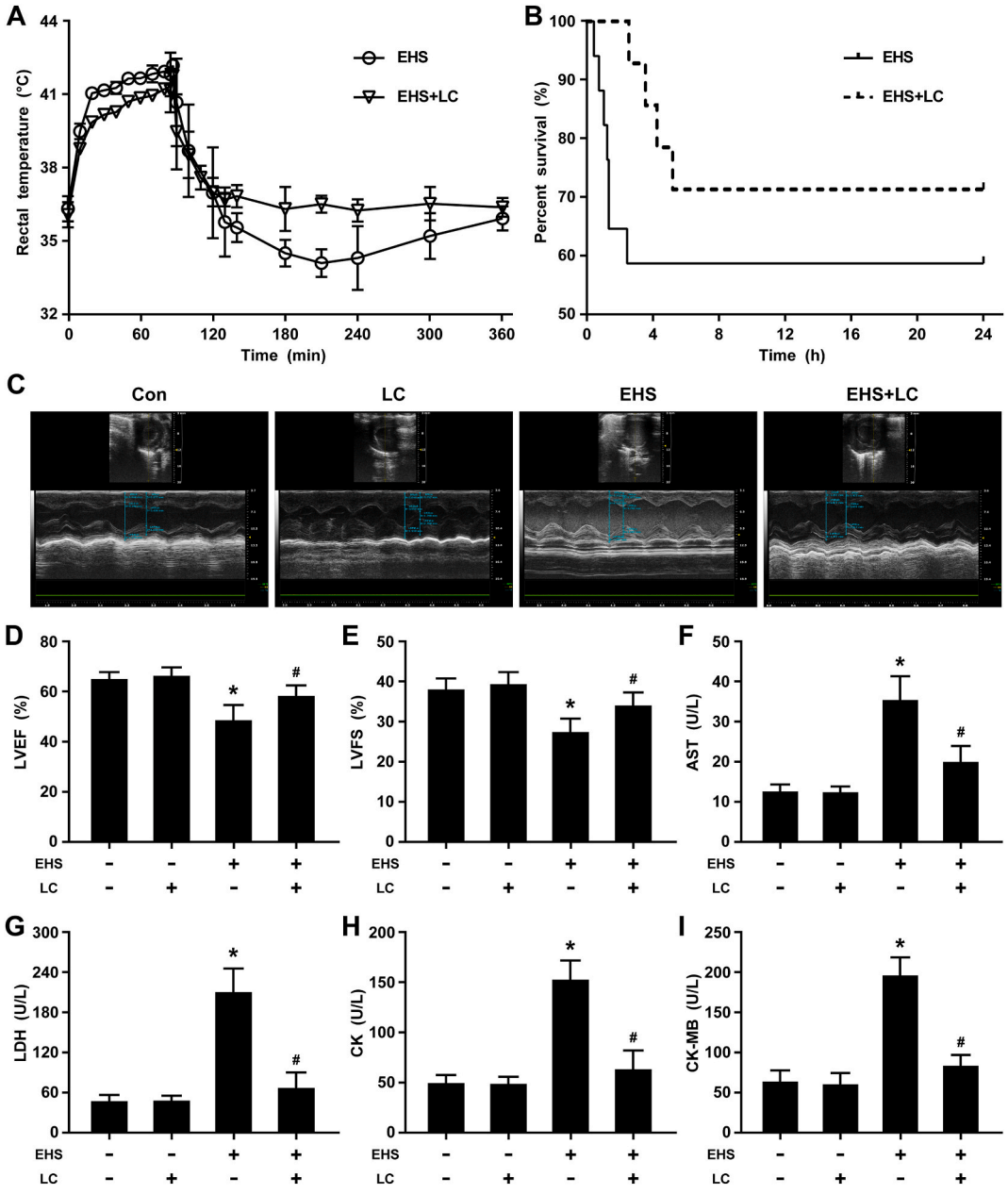


Fig. 1. The protective role of LC against EHS-induced cardiac dysfunction and mortality. Rats aged 8 weeks old were selected and then subjected to EHS with or without LC pretreatment. **(A)** Rectal temperature during EHS. **(B)** Survival curves of EHS rats and LC pre conditioned EHS rats. **(C)** Representative echocardiographic images from each group at 2 h after EHS onset. **(D)** Quantitative analysis of left ventricular ejection fraction (LVEF) among the different groups. **(E)** Quantitative analysis of left ventricular fraction shortening (LVFS) among the different groups. Serum aspartate aminotransferase (AST) **(F)**, lactate dehydrogenase (LDH) **(G)**, creatine kinase (CK) **(H)**, and creatine kinase myocardial band (CK-MB) **(I)** levels at 2 h after EHS onset in control and various treatment groups of rats. The data are representative of three independent experiments using 15 rats per group for (A) and (B), using 6 rats per group for (C). For (D)–(I), the data are presented as the means ± SEMs of three independent experiments using 6 rats per group. $^{*}P < 0.05$ vs the sham-treated control group; $^{\#}P < 0.05$ vs the EHS group.

the following primary antibodies purchased from Cell Signaling Technology: B-cell lymphoma-2 (BCL-2), BCL-2-associated X (BAX), cleaved caspase-3, C/EBP homologous protein (CHOP), phosphorylated protein kinase RNA-like endoplasmic reticulum kinase (p-PERK) Thr980, phosphorylated eukaryotic translation initiation factor 2α (p-eIF2α) Ser51, and spliced X-box binding protein 1 (XBP-1s) (all used at 1:1000 dilution; Danvers, MA, USA), and antibodies obtained from Abcam: glucose regulatory protein 78 (GRP78) and activating transcription factor 6 (ATF6) (both used at 1:2000 dilution, Cambridge, MA, USA). Afterwards, the membranes were washed and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Zhongshan Goldenbridge Biotechnology (Zsbio), Beijing, China) for 1 h at room temperature. After incubation, the membranes were reacted with enhanced chemiluminescence reagent (Bio-Rad, Hercules, CA, USA), and the signal was detected using a ChemiDoc MP gel imaging system (Bio-Rad). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH, 1:1000, Cell Signaling Technology) was used as an internal control. Relative band densities were determined by densitometric analysis using Image Lab software (Bio-Rad).

2.11. Statistical analysis

All experiments and data analyses were conducted in a blinded manner. Statistical analyses were performed using IBM SPSS Statistics software (Version 22.0, IBM Corp., Armonk, NY, USA). The data are expressed as the means ± SEMs. The normality of the data was verified by the Kolmogorov-Smirnov test before further analysis. Significant differences between the groups were assessed by one- or two-way ANOVA followed by Tukey's test. Statistical significance was established at $P < 0.05$.

3. Results

3.1. LC improves cardiac dysfunction and mortality in a rat model of EHS

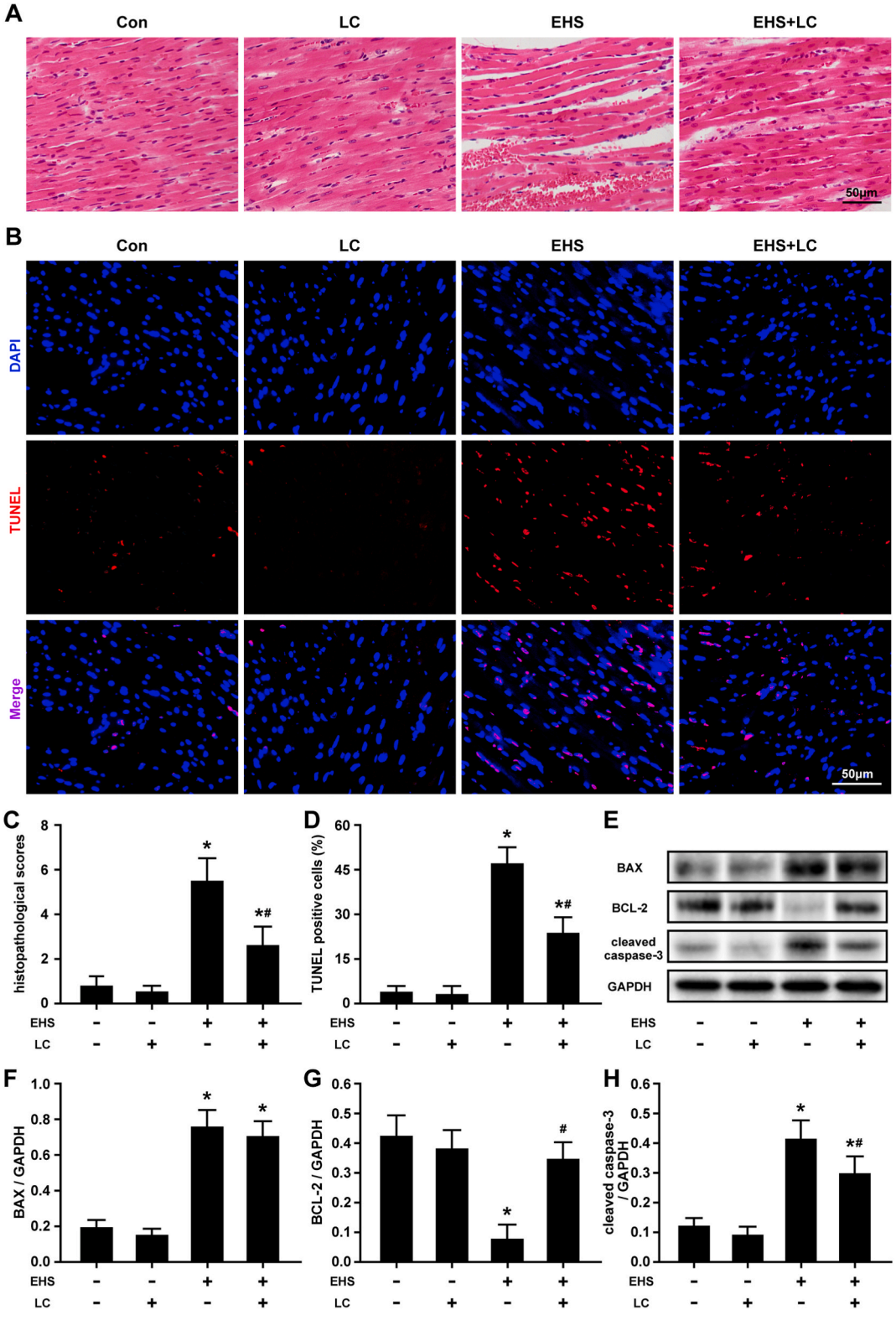
To determine the effects of LC on rectal temperature (Tre), mortality, echocardiographic indices, and myocardial enzymes, Sprague–Dawley normal control rats and LC-pretreated rats were subjected to EHS or the sham treatment. A biphasic Tre profile characterized by hypothermia transition was observed in EHS rats, whereas all LC-pretreated rats exhibited a steady decline of Tre to the baseline value with no observable hypothermia phase (Fig. 1 A). After 24 h of recovery, the mortality rate was as low as 26.7 % (4/15) for the EHS + LC group and 46.7 % (7/15) for the EHS group (Fig. 1 B). The threshold 2-h recovery time after the onset of EHS (2 h after EHS onset) with prominent pathophysiological outcomes was employed in the present study. Echocardiographic examination showed LC pretreatment dramatically reversed the decreased LVEF (Fig. 1C and D) and LVFS (Fig. 1C and E) 2 h after EHS onset. In contrast, rats treated with LC alone showed no significant changes in the levels of LVEF and LVFS, compared with the control rats (Fig. 1C–E). Serum levels of AST (Fig. 1 F), LDH (Fig. 1 G), CK (Fig. 1H), and CK-MB (Fig. 1 I) were markedly increased in EHS rats, compared with the control rats. Moreover, the increased levels of AST, LDH, CK, and CK-MB were significantly reversed by LC treatment (Fig. 1F–I). These results indicate that LC can effectively improve cardiac dysfunction and mortality in the present rat model of EHS.

3.2. LC protects against cardiac injury and cardiomyocyte apoptosis in EHS rats

A thorough cardiac assessment requires a correlation between clinical and histopathological findings [25]. Besides the application of echocardiography and the measurement of myocardial enzymes, pathological examination showed that EHS treatment resulted in an irregular shape or unclear boundary of cardiac myocytes, congested blood vessel, and infiltration of the injured myocardium with lymphocytes (Fig. 2 A). Moreover, LC treatment significantly improved histopathological signs of myocardial injury 2 h after EHS onset (Fig. 2 A). Meanwhile, the myocardial histopathological scores also showed similar trends of changes (Fig. 2C). To determine whether apoptosis mechanisms contribute to this injury caused by EHS, myocardial sections were labeled with TUNEL. TUNEL staining showed that the number of TUNEL-positive cells in EHS rats was increased compared with the control rats (Fig. 2 B). In addition, statistical analysis revealed that the numbers of TUNEL-positive cells were significantly lower in the EHS + LC rats than that in the EHS rats (Fig. 2 D). Furthermore, immunoblotting analyses revealed a significant increase in the expression of BAX (Fig. 2E and F) and cleaved caspase-3 (Fig. 2 E and H), and a significant decrease in BCL-2 expression (Fig. 2 E and G) in EHS rats. As expected, the expression of BCL-2 and cleaved caspase-3 was significantly reversed in EHS + LC rats (Fig. 2 E, G, and H). In contrast, the expression of BAX was not significantly reversed (Fig. 2 E and F). Collectively, these results indicate that the inhibition of cardiomyocyte apoptosis may be linked to the protection of LC against EHS-induced cardiac injury.

3.3. LC alleviates endoplasmic reticulum stress and ultrastructural damage in EHS rats

Considering the detected alterations of apoptosis effector caspase-3 and the apoptosis-associated proteins BAX and BCL-2, we assessed the effects of LC treatment on the expression of ERS-related apoptosis modulator and sensor, such as CHOP and GRP78. It was also found that EHS treatment significantly increased the expression of GRP78 and CHOP (Fig. 3A–C). As expected, no differences were found for the expression of GRP78 and CHOP between control rats and LC-treated rats (Fig. 3A–C). Consistent with the alterations in the expression of cleaved caspase-3 and BCL-2 (Fig. 2E–G, and H), LC treatment significantly inhibited the upregulation of GRP78 and CHOP protein expression 2 h after EHS onset (Fig. 3A–C). Clearly, TEM examination showed that rats subjected to EHS exhibited ultrastructural damage manifested by disorganized ER, extremely swollen or vacuolated mitochondria with broken crista, and altered connection of ER and mitochondria (Fig. 3 D). In contrast, compared with the EHS-treated rats, ultrastructural alterations in the



(caption on next page)

Fig. 2. The protective role of LC against EHS-induced cardiac injury and cardiomyocyte apoptosis. Rats aged 8 weeks old were selected and then subjected to EHS with or without LC pretreatment. **(A)** Representative HE staining of cardiac sections at 2 h after EHS onset. **(B)** Representative photomicrographs from TUNEL-stained cardiac sections at 2 h after EHS onset. **(C)** Semi-quantitative myocardial histopathological scores were calculated from **(A)**. **(D)** Quantification of TUNEL-positive (TUNEL+) cells was calculated from **(B)**. **(E)** The expression of BAX, BCL-2, and cleaved caspase-3 in total cardiac lysates were detected using Western blotting at 2 h after EHS onset. The densitometric analysis was performed for BAX **(F)**, BCL-2 **(G)**, and cleaved caspase-3 **(H)**. For (A) and (B), the data are representative of three independent experiments using 6 rats per group, 8–10 sections per heart. Scale bar = 50 μm. For (C)–(H), the data are presented as the means ± SEMs of three independent experiments using 6 rats per group. $^{*}P < 0.05$ vs the sham-treated control group; $^{\#}P < 0.05$ vs the EHS group.

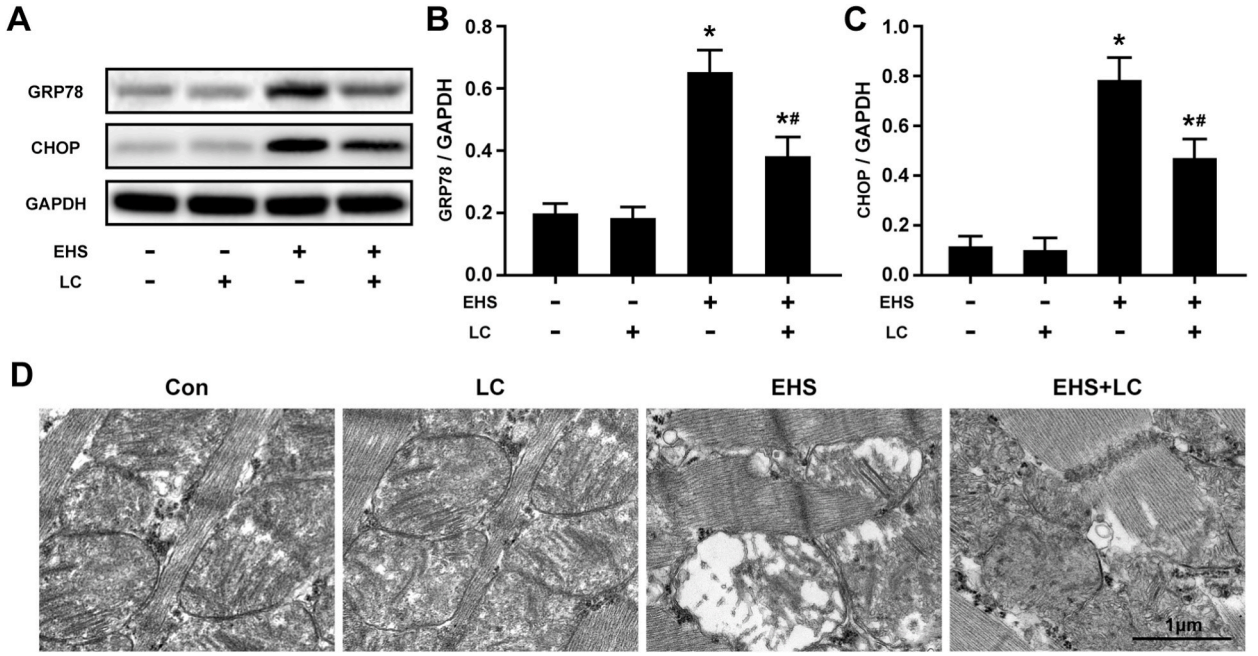


Fig. 3. Effects of LC on endoplasmic reticulum stress and ultrastructural damage in EHS rats. Rats aged 8 weeks old were selected and then subjected to EHS with or without LC pretreatment. **(A)** The expression of GRP78 and CHOP in total cardiac lysates were detected using Western blotting at 2 h after EHS onset. The densitometric analysis was performed for GRP78 **(B)** and CHOP **(C)**. **(D)** Representative TEM images of cardiac cellular structures of rats at 2 h after EHS onset. The data are presented as the means ± SEMs of three independent experiments using 6 rats per group. For (D), scale bar = 1 μm. For (B) and (C), $^{*}P < 0.05$ vs the sham-treated control group; $^{\#}P < 0.05$ vs the EHS group.

cardiac tissues of rats were attenuated by LC treatment (Fig. 3 D). These results indicate that LC can regulate ERS and ultrastructural damage in response to EHS-induced myocardial injury.

3.4. LC attenuates myocardial apoptosis and ultrastructural damage via inhibiting ERS

To validate the effects of ERS on myocardial apoptosis and mitochondrial damage, rats were pretreated with or without an ERS antagonist 4-PBA and then subjected to EHS. It was found that 4-PBA pretreatment reversed not only the altered expression of cleaved caspase-3 and BCL-2 caused by EHS (Fig. 4A–C, and D), but also the changed expression of GRP78 and CHOP (Fig. 4A–E, and F). Additionally, 4-PBA treatment was found to attenuate the ultrastructural changes of ER and mitochondria (Fig. 4 G). These results confirmed that pharmacological inhibition of ERS contributed to the attenuation of myocardial apoptosis and mitochondrial damage. Considering the similar protective roles of LC and 4-PBA in EHS-induced myocardial injury, we further determined whether LC reduces apoptosis and attenuates ultrastructural damage via regulating ERS. TG, which was used to specifically induce ERS, was employed in this study. Western blotting analysis showed that protein expression levels of GRP78 (Fig. 5 A and E), CHOP (Fig. 5 A and F), cleaved caspase-3 (Fig. 5 A and D), and BAX (Fig. 5A and B) were enhanced by TG, while the expression of BCL-2 (Fig. 5 A and C) was decreased by TG in EHS + LC rats. Moreover, TEM examination indicated that the attenuation effect of LC on EHS-induced ultrastructural damage was dramatically prevented by TG (Fig. 5 G).

3.5. LC alleviates ERS via inhibiting PERK signaling pathway

The aforementioned findings showed that LC attenuates myocardial apoptosis and ultrastructural damage through alleviating ERS. As with the gradually reported effects of heat stress on ER stress pathways [11–13], subsequently, the representative signaling

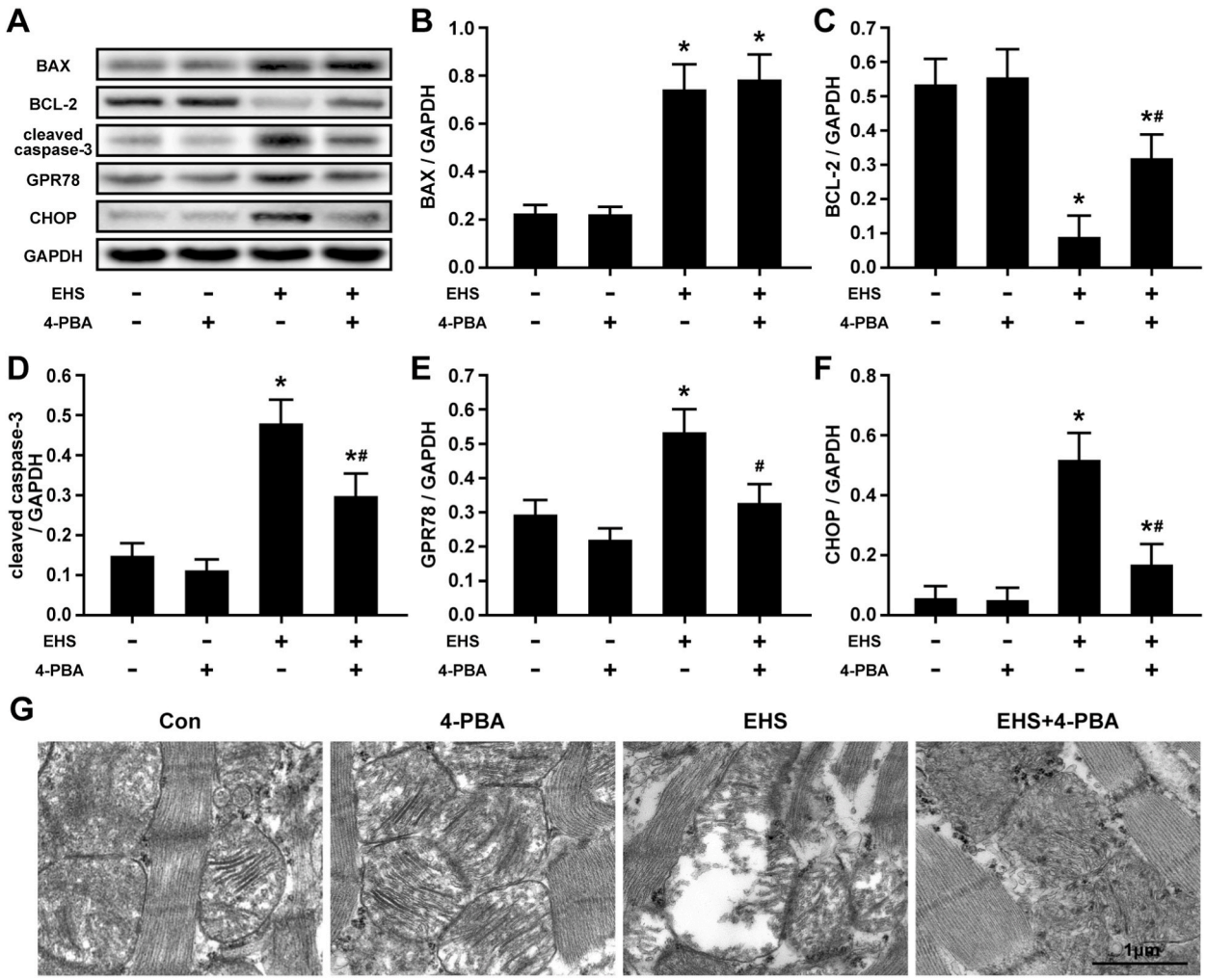


Fig. 4. Effects of ERS inhibition on myocardial apoptosis and ultrastructural damage in EHS rats. Rats aged 8 weeks old were selected and then subjected to EHS with or without 4-Phenyl butyric acid (4-PBA, a potent ERS inhibitor) pretreatment. **(A)** The expression of BAX, BCL-2, cleaved caspase-3, GRP78, and CHOP in total cardiac lysates were detected using Western blotting at 2 h after EHS onset. The densitometric analysis was performed for BAX **(B)**, BCL-2 **(C)**, cleaved caspase-3 **(D)**, GRP78 **(E)**, and CHOP **(F)**. **(G)** Representative TEM images of cardiac cellular structures of rats at 2 h after EHS onset. The data are presented as the means ± SEMs of three independent experiments using 6 rats per group. For (G), scale bar = 1 μm. For (B)–(F), $^{*}P < 0.05$ vs the sham-treated control group; $^{\#}P < 0.05$ vs the EHS group.

effectors, p-PERK, p-eIF2α, ATF6, and XBP-1s, were determined using the Western blotting analysis to further reveal the preventive mechanism of LC underlying EHS-induced cardiac injury and cardiomyocyte apoptosis. Western blotting analysis showed that the expression of p-PERK and p-eIF2α was significantly increased in EHS rats (Fig. 6A–C). LC treatment dramatically suppressed the upregulation of p-PERK and p-eIF2α 2 h after EHS onset (Fig. 6A–C), showing similar affected pattern to CHOP (Fig. 3 A and C). In contrast, the other two ERS-response effectors, ATF6 and XBP-1s, showed different changes by LC treatment in EHS rats. Compared with the control rats, a significant increase in XBP-1s expression, but slightly increased ATF6 expression, were found in EHS rats (Fig. 6 A, D, and E). However, EHS-induced expression of ATF6 and XBP-1s was not affected by LC in the sham or EHS rats (Fig. 6 A, D, and E). Overall, these data suggest that PERK-eIF2α-CHOP pathway may be the predominant therapeutic target of LC against EHS-induced cardiac injury and cardiomyocyte apoptosis, resulting in the improvement of cardiac dysfunction and mortality in EHS.

4. Discussion

Regulation of ERS play a crucial role in pharmacological intervention of cardiomyocyte apoptosis and cardiac injury for a number of cardiovascular diseases [26]. In the present study, we observed a biphasic Tre profile, a relatively high mortality rate, and structural and functional impairments of the heart in the present rat model of EHS. Notably, as a beneficial intervention, LC treatment significantly attenuated histopathological and ultrastructural alterations of myocardial injury via inhibiting the upregulation of ERS-related apoptosis modulator and sensor. Experiments on the effects of pharmacological induction and inhibition of ERS provided further

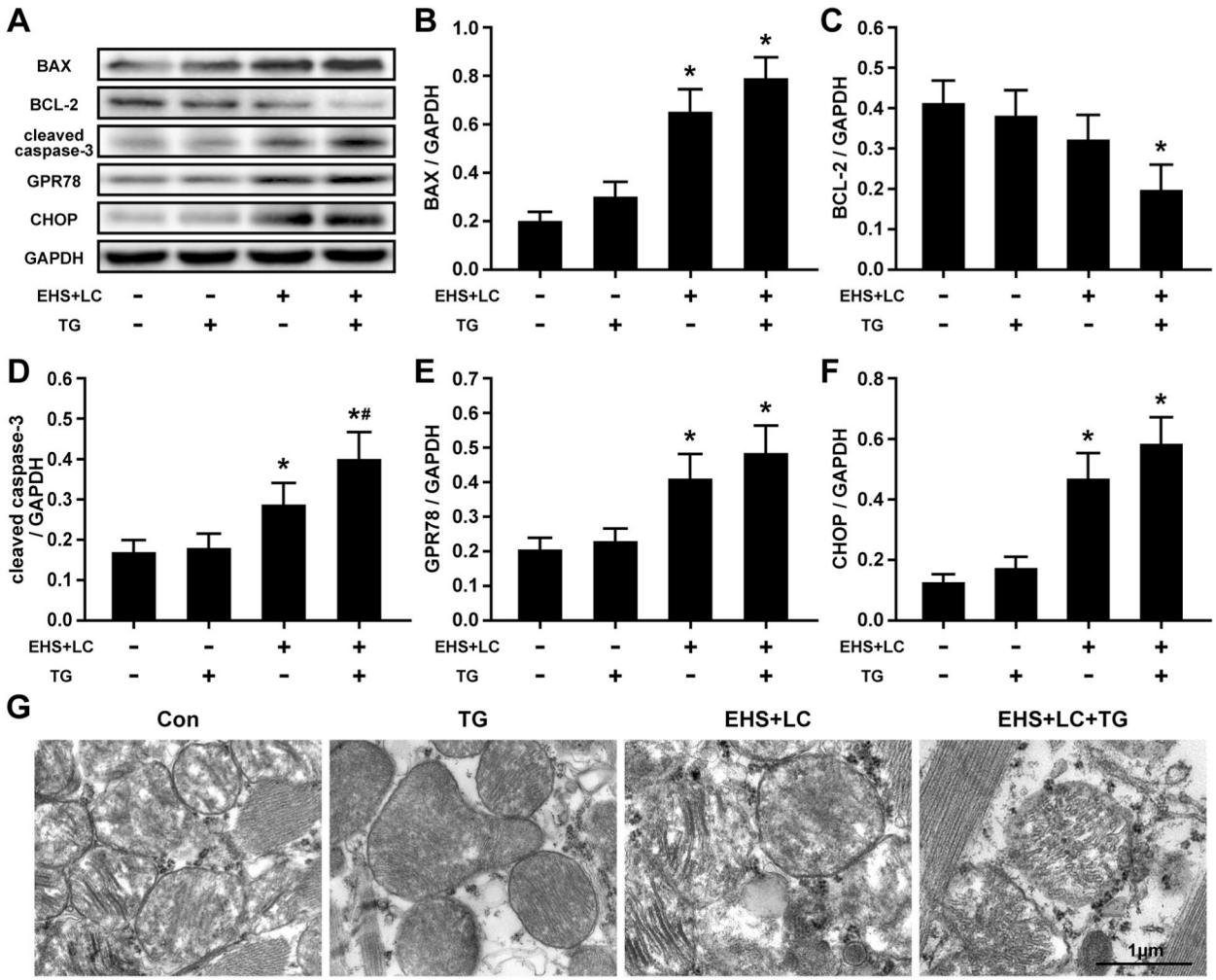


Fig. 5. Effects of ERS induction on myocardial apoptosis and ultrastructural damage in EHS rats with LC preconditioning. Rats aged 8 weeks old were selected and then subjected to LC + EHS with or without thapsigargin (TG, a known ERS activator) pretreatment. **(A)** The expression of BAX, BCL-2, cleaved caspase-3, GRP78, and CHOP in total cardiac lysates were detected using Western blotting at 2 h after EHS onset. **(B)** The densitometric analysis was performed for BAX **(B)**, BCL-2 **(C)**, cleaved caspase-3 **(D)**, GRP78 **(E)**, and CHOP **(F)**. **(G)** Representative TEM images of cardiac cellular structures of rats at 2 h after EHS onset. The data are presented as the means ± SEMs of three independent experiments using 6 rats per group. For (G), scale bar = 1 μm. For (B)–(F), $^{*}P < 0.05$ vs the sham-treated control group; $^{\#}P < 0.05$ vs the LC-pretreated EHS group.

evidence that LC reduces myocardial apoptosis and attenuates ultrastructural damage of ER and mitochondria via partially regulating ERS. These data are consistent with previous finding demonstrating that LC prevents myocardial apoptosis [17]. Moreover, LC treatment dramatically suppressed the upregulation of p-PERK, p-eIF2α, and CHOP, but not the expression of ATF6 and XBP-1s in EHS rats. Taken together, these results indicate that LC reverses EHS-induced myocardial apoptosis and ultrastructural damage via the regulation of PERK, not ATF6 and XBP-1s, linking to the potential for therapeutic intervention.

Despite effective treatment strategies of EHS are still limited, emerging evidence now supports the deleterious effect of heat on the heart and the exploration of cardioprotection in heatstroke management [3,27]. Several clinical and experimental studies have shown that EHS can induce cardiovascular overload and myocardial injury [28,29]. Recent experimental data further support myocardial structure and functional alterations such as EHS-induced myocardial damage, pathological echocardiography, myocardial fibrosis, and hypertrophy, in a preclinical model of EHS [28]. Consistently, in this study, we observed histopathological signs of myocardial injury, echocardiographic indices of cardiac dysfunction, and a high mortality rate in the present rat model of EHS. Notably, although a dearth of clinical validation exists regarding the cardioprotective therapies for EHS management, regulation of cellular apoptosis, oxidative stress, and inflammation in animal models of heatstroke has been clearly reported to possess potential cardioprotective capabilities using numerous active compounds, including myricetin [30], salidroside [31], melatonin [32], and quercetin [33]. Consequently, we also found a similar protective effects of L-carnitine (LC), a quaternary ammonium compound, on myocardial structure and function in EHS rats. However, there has been no head-to-head estimated pharmacological effects of different interventions and the possible mechanistic differences. In agreement with numerous studies, we focused our study on the biological consequences in the realm of

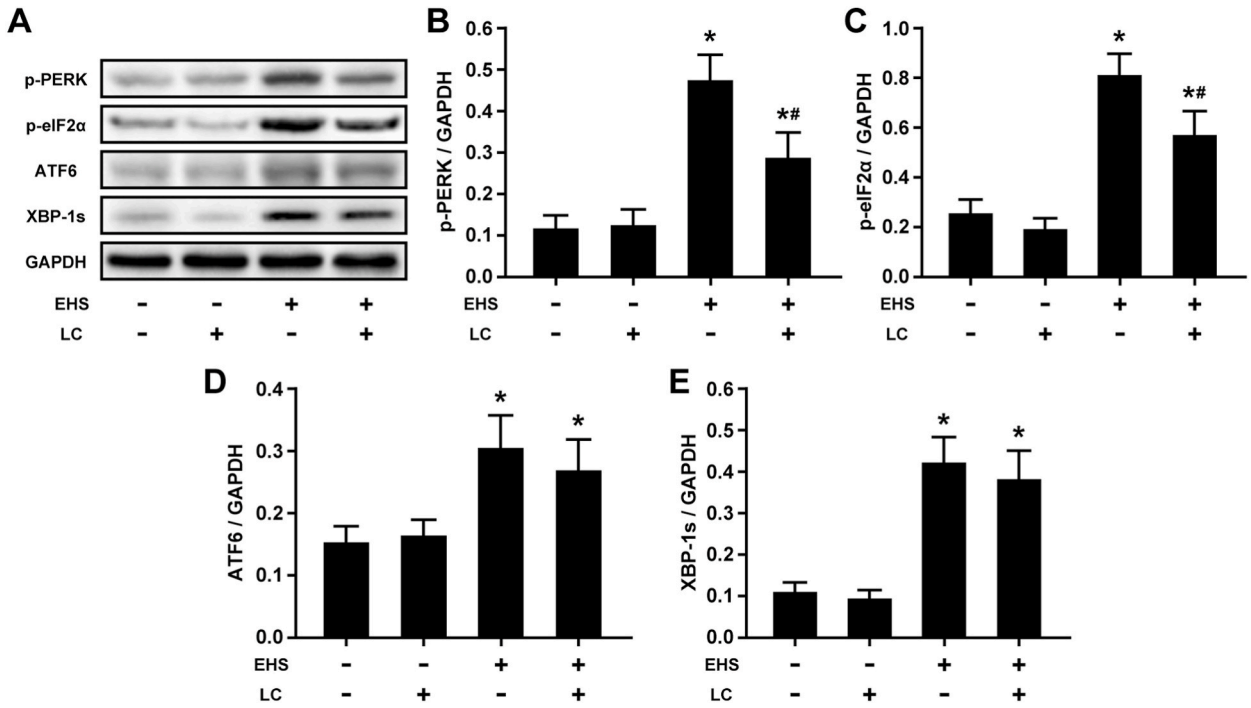


Fig. 6. Effects of LC on endoplasmic reticulum stress pathways in EHS rats. Rats aged 8 weeks old were selected and then subjected to EHS with or without LC pretreatment. **(A)** The phosphorylation of PERK and eIF2α and the expression of ATF6 and XBP-1s in total cardiac lysates were detected using Western blotting at 2 h after EHS onset. The densitometric analysis was performed for the phosphorylation of PERK **(B)** and eIF2α **(C)** and the expression of ATF6 **(D)** and XBP-1s **(E)**. The data are presented as the means ± SEMs of three independent experiments using 6 rats per group. For (B)–(E), $^{*}P < 0.05$ vs the sham-treated control group; $^{\#}P < 0.05$ vs the EHS group.

animal studies, which needed further clinical validation. Here, our results combined with previous studies suggest that besides the basic and advanced cardiac life support, specific pharmacologic cardioprotective interventions may be helpful for preventing and managing EHS.

It is well known that LC plays an important role in averting a wide range of cardiac problems by exerting anti-oxidative, anti-inflammatory, and anti-apoptotic effects [34]. Several experimental and clinical models indicate that LC has a significant cardioprotective action against cardiac dysfunction [35], cardiomyocytes apoptosis [36], inflammatory response [37], oxidative stress [38], mitochondrial dysfunction [39], contractile dysfunction [40]. In line with these findings, elevated serum enzymes such as AST, LDH, CK, and CK-MB were significantly increased in the present rat model of EHS, reflecting impairment in cardiac function. Although AST and LDH enzymes were ubiquitously expressed in a wide variety of tissues and lacked specificity for myocardial injury, they could serve as decent systems biomarker candidates when combined with other lab results [41]. In addition, the elevated serum levels of CK and CK-MB further improve specificity for the initial evaluation of cardiac damage [42]. Moreover, abnormal diastolic echocardiographic indices along with the presence of simultaneous alterations of AST, LDH, CK, and CK-MB after EHS, enhanced the detection of myocardial injury and cardiac dysfunction. Remarkably, we delineated the strong modulatory capabilities of LC against echocardiographic abnormalities and the aforementioned enzymes evoked by EHS in adult male rats. Similar to our findings, decreased serum markers and increased LVEF were observed following LC treatment during myocardial ischaemia–reperfusion injury in patients with rheumatic valvular heart disease under cardiopulmonary bypass surgery [35]. Notably, the choice of dosage of LC is comparable to the one recommended for infant/adult (standard reference material 1849 nutritional formula) (136±14 mg/kg), and also based on the use of LC (100 mg/kg) in rat models of disease management [15], herein, displaying a greater effect in rescuing EHS-induced myocardial injury. A meta-analysis showed that LC supplementation reduce one-year mortality in septic patients, whose end-stage clinical features closely resemble that of heatstroke, however, the clinical implications of similar dosages of LC as 6–18 gr over a 12-h period limits the possibility of determining the effective dose of LC [43]. In addition, no estimates based on safety and considerable differences in the strength of design and performance of clinical trials, have been made of the possible dose-dependent impact of LC supplementation on the potential risk factors in patients with EHS. Therefore, it is possible that LC with an optimal therapeutic range identified in further experiments may provide critical aid to ameliorate myocardial injury and cardiac dysfunction in EHS.

Theoretically, aggravation of apoptosis, inflammatory response, oxidative stress, and autophagy may be associated with the underlying mechanisms of myocardial injury and cardiac dysfunction [44–46]. Among these, apoptosis, a major form of cell death, is considered to be the most prominent affliction of cardiomyocytes, which may have more deleterious effects on myocardial injury and cardiac dysfunction [47]. Indeed, we found that increased number of TUNEL-positive cells, increased expression of cleaved caspase-3 and BAX, and decreased BCL-2 expression were observed in the myocardium of EHS rats. In an appropriately extended sense, the

inhibition of apoptosis promises to be an extraordinarily important target for therapeutic intervention [48]. Recently, Mansour et al. indicated that LC abrogated cardiac damage and apoptosis induced by imatinib, a tyrosine kinase inhibitor used to treat chronic myeloid leukemia and gastrointestinal stromal tumors [49]. Moreover, LC is considered to have good therapeutic potential for myocardial infarction, which is probably attributable to the inhibition of BAX/BCL-2-mediated apoptosis [17]. In the present study, LC treatment was found to decrease TUNEL-positive cells, attenuate the expression of cleaved caspase-3 but not BAX, and increased BCL-2 expression in EHS rats. Our results combined with previous studies, suggest, in partial, the involvement of apoptosis inhibition in the cardioprotective effects of LC against EHS-induced myocardial injury.

Various mechanisms of apoptosis induction are related to the plasma membrane and membrane organelles, such as endoplasmic reticulum (ER), mitochondria, Golgi apparatus, lysosomes, and nucleus [50]. Among these, ER is the largest organelle in the cell and is rapidly respond to environment sensing, which is critical for stress-induced damage. Over the past few decades, a strong ER stress response in different animals and tissues has been observed in several studies of various fields, such as heat stress, heavy metal toxicity, and ischemic stroke [11,51,52]. In this study, pathological examination showed irregular shape or unclear boundary, and TEM examination showed disorganized ER, extremely swollen or vacuolated mitochondria with broken crista, and altered connection of ER and mitochondria in EHS rats, indicating the crucial roles of ER, mitochondria, and their communication in controlling apoptosis [53]. Meanwhile, EHS significantly increased the expression of ERS-related apoptosis modulator and sensor, CHOP and GRP78. Moreover, LC treatment significantly reversed these alterations induced by EHS. Notably, therapeutic strategies targeting ERS have attracted significant research interest [54]. Previous studies have reported that LC reduces ERS and cell apoptosis in different types of cells, including renal tubular cells [23] and in neuroblastoma SH-SY5Y cells [55]. Subsequently, our data also showed that pharmacological inhibition of ERS by 4-PBA significantly reversed not only the alterations of regulatory proteins for apoptosis and ERS, but also the ultrastructural changes of ER and mitochondria, exhibiting similar cardioprotective properties of LC under EHS conditions. In contrast, pharmacological induction of ERS by TG dramatically abolished the cardioprotective effects of LC on EHS-induced ultrastructural damage and ERS- and apoptosis-related proteins. Thus, these results suggest a potent regulation of ERS-associated apoptosis by the action of LC in EHS rats.

Mechanismly, apoptosis is closely associated with the ERS pathway involving activation of PERK, ATF6, and XBP-1s (an IRE1 executioner) [56]. In this study, we found that EHS significantly increased the expression of p-PERK, p-eIF2α, and XBP-1s, but slightly increased ATF6 expression in adult rats. Conversely, LC treatment dramatically suppressed the upregulation of p-PERK and p-eIF2α but not XBP-1s and ATF6 in EHS rats. Our observation is supported by the latest report demonstrated the involvement of the PERK/elF2α/CHOP signaling pathway in apoptosis and neuronal damage following heat stress [57]. Similarly, ursolic acid, a natural compound, prevented lung dysfunction by inactivating PERK-eIF2α-CHOP pathway but not ATF6 and IRE-1α pathways, in mice sacrificed 2 h after heat stress [58]. In addition, a recent study reported that LC eliminated the upregulation of IRE1α (as a representative biomarker of ER stress) in the renal tubular cells treated with perfluorooctanesulfonate for 24 h [59]. Thus, the heterogeneous regulation of ER stress response pathways by LC may be largely attributed to the different parameters and recovery periods of stress-state, as well as the different roles of ER stress pathways in cell survival and cell death. These studies also inspire future research attention that aim to explore the molecules that function as inhibitors of apoptotic ER stress response pathway. Regardless of the detailed mechanism, the data presented in this study may assist future studies that aim to determine the therapeutic potential of LC against EHS.

5. Conclusion

Our preliminary results indicate that L-carnitine exhibit a cardioprotective property at least in part via PERK/elF2α/CHOP signaling pathway in the regulation of ERS-associated apoptosis in the present rat model of EHS. L-carnitine might be a therapeutic option to ameliorates myocardial injury for EHS and other cardiovascular diseases.

CRediT authorship contribution statement

Bo-Yi Zhang: Writing – original draft, Software, Methodology, Investigation, Data curation. **Gen-Lin He:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ze-Ze Wang:** Validation, Software, Resources, Methodology, Formal analysis, Data curation. **Huan Zhou:** Validation, Supervision, Resources, Methodology, Investigation. **Xue-Yan Huang:** Visualization, Validation, Software, Data curation. **Ting-Ting Shen:** Supervision, Software, Investigation, Formal analysis. **Xiao-Qian Liu:** Validation, Software, Data curation. **Yi-Shan Liu:** Software, Methodology. **Zhen Luo:** Visualization, Validation, Formal analysis. **Ping Li:** Validation, Formal analysis, Data curation. **Yu-Long Tan:** Validation, Methodology. **Xue Luo:** Validation, Software. **Xue-Sen Yang:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization.

Data availability

Data will be made available on request.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge biomedical analysis center and the affiliated first hospital of Army Medical University, China for providing necessary equipment and technical assistance to complete this study.

References

[1] Y. Epstein, D.L. Longo, R. Yanovich, Heatstroke, N. Engl. J. Med. 380 (25) (2019) 2449–2459.
[2] C.K. Garcia, L.I. Renteria, G. Leite-Santos, L.R. Leon, O. Laitano, Exertional heat stroke: pathophysiology and risk factors, BMJ Med 1 (1) (2022) e000239.
[3] R. Xia, M. Sun, Y. Li, J. Yin, H. Liu, J. Yang, J. Liu, Y. He, B. Wu, G. Yang, J. Li, The pathogenesis and therapeutic strategies of heat stroke-induced myocardial injury, Front. Pharmacol. 14 (2024) 1286556.
[4] L. Zhong, J. Ji, C. Wang, Z. Liu, Clinical characteristics and risk factors of male exertional heatstroke in patients with myocardial injury: an over 10-year retrospective cohort study, Int. J. Hyperther. 38 (1) (2021) 970–975.
[5] J.-C. Wang, W.-C. Chien, P. Chu, C.-H. Chung, C.-Y. Lin, S.-H. Tsai, The association between heat stroke and subsequent cardiovascular diseases, PLoS One 14 (2) (2019) e0211386.
[6] C. Rublee, C. Dresser, C. Giudice, J. Lemery, C. Sorensen, Evidence-based heatstroke management in the emergency department, West. J. Emerg. Med. 22 (2) (2021) 186–195.
[7] J. Patel, N. Boyer, K. Mensah, S. Haider, O. Gibson, D. Martin, E. Walter, Critical illness aspects of heatstroke: a hot topic, J. Intensive Care Soc. 24 (2) (2023) 206–214.
[8] Y. Cao, Z. Liu, W. Xiao, Z. Gu, G. Xiao, F. Yuan, F. Chen, Y. Pei, H. Li, L. Su, 4-Phenylbutyrate prevents endoplasmic reticulum stress-mediated apoptosis induced by heatstroke in the intestines of mice, Shock 54 (1) (2020) 102–109.
[9] Z.T. Gu, L. Li, F. Wu, P. Zhao, H. Yang, Y.S. Liu, Y. Geng, M. Zhao, L. Su, Heat stress induced apoptosis is triggered by transcription-independent p53, Ca^{2+} dyshomeostasis and the subsequent Bax mitochondrial translocation, Sci. Rep. 5 (2015) 11497.
[10] D.A. Bettaieb, Averill-Bates, Thermotolerance induced at a fever temperature of 40 C protects cells against hyperthermia-induced apoptosis mediated by death receptor signalling, Biochem. Cell. Biol. 86 (6) (2008) 521–538.
[11] Y. Yang, C. Li, X. Xiang, Z. Dai, J. Chang, M. Zhang, H. Cai, H. Zhang, M. Zhang, Y. Guo, Z. Wu, Ursolic acid prevents endoplasmic reticulum stress-mediated apoptosis induced by heat stress in mouse cardiac myocytes, J. Mol. Cell. Cardiol. 67 (2014) 103–111.
[12] H. Oghbaei, L. Hosseini, F. Farajdokht, S. Rahigh Aghsan, A. Majdi, S. Sadigh-Eteghad, S. Sandoghchian Shotorbani, J. Mahmoudi, Heat stress aggravates oxidative stress, apoptosis, and endoplasmic reticulum stress in the cerebellum of male C57 mice, Mol. Biol. Rep. 48 (8) (2021) 5881–5887.
[13] Y. Liu, H. Sakamoto, M. Adachi, S. Zhao, W. Ukai, E. Hashimoto, M. Hareyama, T. Ishida, K. Imai, Y. Shinomura, Heat stress activates ER stress signals which suppress the heat shock response, an effect occurring preferentially in the cortex in rats, Mol. Biol. Rep. 39 (2012) 3987–3993.
[14] H.-F. Zeng, J. Xu, X.-L. Wang, S.-J. Li, Z.-Y. Han, Nicotinamide mononucleotide alleviates heat stress-induced oxidative stress and apoptosis in BMECs through reducing mitochondrial damage and endoplasmic reticulum stress, Ecotoxicol. Environ. Saf. 235 (2022) 113441.
[15] A.H. Alhasaniah, L-carnitine: nutrition, pathology, and health benefits, Saudi J. Biol. Sci. 30 (2) (2023) 103555.
[16] S. Busquets, R. Serpe, M. Toledo, A. Betancourt, E. Marmonti, M. Orpí, F. Pin, E. Capdevila, C. Madeddu, F.J. López-Soriano, G. Mantovani, A. Macciò, J. M. Argilés, L-carnitine: an adequate supplement for a multi-targeted anti-wasting therapy in cancer, Clin. Nutr. 31 (6) (2012) 889–895.
[17] H.-R. Li, X.-M. Zheng, Y. Liu, J.-H. Tian, J.-J. Kou, J.-Z. Shi, X.-B. Pang, X.-M. Xie, Y. Yan, L-carnitine alleviates the myocardial infarction and left ventricular remodeling through Bax/Bcl-2 signal pathway, Cardiovasc. Ther. 2022 (2022) 9615674.
[18] D. Wu, H. He, J. Chen, S. Yao, H. Xie, W. Jiang, X. Lv, W. Gao, L. Meng, X. Yao, L-carnitine reduces acute lung injury via mitochondria modulation and inflammation control in pulmonary macrophages, Braz. J. Med. Biol. Res. 56 (2023) e12830.
[19] M.I. Hiskens, K.M. Li, A.G. Schneiders, A.S. Fenning, Repetitive mild traumatic brain injury-induced neurodegeneration and inflammation is attenuated by acetyl-L-carnitine in a preclinical model, Front. Pharmacol. 14 (2023) 1254382.
[20] S.A. Salama, H.H. Arab, H.A. Omar, H.S. Gad, G.M. Abd-Allah, I.A. Maghrabi, M.M. Al Robaian, L-carnitine mitigates UVA-induced skin tissue injury in rats through downregulation of oxidative stress, p38/c-Fos signaling, and the proinflammatory cytokines, Chem. Biol. Interact. 285 (2018) 40–47.
[21] F. Vacante, P. Senesi, A. Montesano, A. Frigerio, L. Luzi, I. Terruzzi, L-Carnitine: an antioxidant remedy for the survival of cardiomyocytes under hyperglycemic condition, J. Diabetes Res. 2018 (2018) 4028297.
[22] C.-Y. Mao, H.-B. Lu, N. Kong, J.-Y. Li, M. Liu, C.-Y. Yang, P. Yang, Levocarnitine protects H9c2 rat cardiomyocytes from H_2O_2-induced mitochondrial dysfunction and apoptosis, Int. J. Med. Sci. 11 (11) (2014) 1107–1115.
[23] Y.-C.G. Lee, H.-C. Chou, Y.-T. Chen, S.-Y. Tung, T.-L. Ko, B. Buyandelger, L.-L. Wen, S.-H. Juan, L-carnitine reduces reactive oxygen species/endoplasmic reticulum stress and maintains mitochondrial function during autophagy-mediated cell apoptosis in perfluorooctanesulfonate-treated renal tubular cells, Sci. Rep. 12 (1) (2022) 4673.
[24] J.-F. Yi, G.-L. He, J. Yang, Z. Luo, X. Luo, X.-S. Yang, Heat acclimation regulates the autophagy-lysosome function to protect against heat stroke-induced brain injury in mice, Cell. Physiol. Biochem. 41 (2017) 101–114.
[25] S. Pichler Sekulic, M. Sekulic, Neovascularization of native cardiac valves, and correlation with histopathologic, clinical, and radiologic features, Cardiovasc. Pathol. 69 (2024) 107605.
[26] S. Wang, P. Binder, Q. Fang, Z. Wang, W. Xiao, W. Liu, X. Wang, Endoplasmic reticulum stress in the heart: insights into mechanisms and drug targets, Br. J. Pharmacol. 175 (8) (2018) 1293–1304.
[27] Y. Desai, H. Khraishah, B. Alahmad, Heat and the heart, Yale J. Biol. Med. 96 (2) (2023) 197–203.
[28] C.-M. Chao, L.-Y. Wang, C.-C. Huang, W.-T. Chang, L.-Y. Tang, M.-T. Lin, C.-P. Chang, Myocardial structure and functional alterations in a preclinical model of exertional heat stroke, Life Sci. 323 (2023) 121640.
[29] D.W. DeGroot, F.G. O'Connor, W.O. Roberts, Exertional heat stroke: an evidence based approach to clinical assessment and management, Exp. Physiol. 107 (10) (2022) 1172–1183.
[30] X. Lin, C.-H. Lin, R. Liu, C. Li, S. Jiao, X. Yi, M.J. Walker, X.-M. Xu, T. Zhao, P.-C. Huang, G. Sun, Myricetin against myocardial injury in rat heat stroke model, Biomed. Pharmacother. 127 (2020) 110194.
[31] G.-d. Chen, H. Fan, J.-H. Zhu, Salidroside pretreatment protects against myocardial injury induced by heat stroke in mice, J. Int. Med. Res. 47 (10) (2019) 5229–5238.
[32] X. Lin, T. Zhao, C.-H. Lin, D. Zuo, Z. Ye, S. Lin, S. Wen, L. Liu, M.-T. Lin, C.-P. Chang, C.-M. Chao, Melatonin provides protection against heat stroke-induced myocardial injury in male rats, J. Pharm. Pharmacol. 70 (6) (2018) 760–767.
[33] X. Lin, C.-H. Lin, T. Zhao, D. Zuo, Z. Ye, L. Liu, M.-T. Lin, Quercetin protects against heat stroke-induced myocardial injury in male rats: antioxidative and antiinflammatory mechanisms, Chem. Biol. Interact. 265 (2017) 47–54.
[34] Z.-Y. Wang, Y.-Y. Liu, G.-H. Liu, H.-B. Lu, C.-Y. Mao, L-carnitine and heart disease, Life Sci. 194 (2018) 88–97.

[35] M. Li, S. Xu, Y. Geng, L. Sun, R. Wang, Y. Yan, H. Wang, Y. Li, Q. Yi, Y. Zhang, J. Hao, C. Deng, W. Li, L. Xue, The protective effects of L-carnitine on myocardial ischaemia–reperfusion injury in patients with rheumatic valvular heart disease undergoing CPB surgery are associated with the suppression of NF-κB pathway and the activation of Nrf2 pathway, Clin. Exp. Pharmacol. Physiol. 46 (11) (2019) 1001–1012.
[36] L. Zhang, L. Xiu, T. Wang, D. Zhao, Effect of L-carnitine in ameliorating lipopolysaccharide-induced cardiomyocyte injury via MAPK Signaling, Mol. Biotechnol. 66 (1) (2024) 79–89.
[37] T. Emran, N.I. Chowdhury, M. Sarker, A.K. Bepari, M. Hossain, G.M.S. Rahman, H.M. Reza, L-carnitine protects cardiac damage by reducing oxidative stress and inflammatory response via inhibition of tumor necrosis factor-alpha and interleukin-1beta against isoproterenol-induced myocardial infarction, Biomed. Pharmacother. 143 (2021) 112139.
[38] R.A. Al-Eisa, F.A. Al-Salmi, R.Z. Hamza, N.S. El-Shenawy, Role of L-carnitine in protection against the cardiac oxidative stress induced by aspartame in Wistar albino rats, PLoS One 13 (11) (2018) e0204913.
[39] E. Oyanagi, H. Yano, M. Uchida, K. Utsumi, J. Sasaki, Protective action of L-carnitine on cardiac mitochondrial function and structure against fatty acid stress, Biochem. Biophys. Res. Commun. 412 (1) (2011) 61–67.
[40] M. Xue, X. Chen, Z. Guo, X. Liu, Y. Bi, J. Yin, H. Hu, P. Zhu, J. Zhuang, C. Cates, T. Rousselle, J. Li, L-carnitine attenuates cardiac dysfunction by ischemic insults through Akt signaling pathway, Toxicol. Sci. 160 (2) (2017) 341–350.
[41] S. Aydin, K. Ugur, S. Aydin, İ. Sahin, M. Yardim, Biomarkers in acute myocardial infarction: current perspectives, Vasc, Health Risk Manag. 15 (2019) 1–10.
[42] R. Jacob, M. Khan, Cardiac biomarkers: what is and what can be, Indian J. Cardiovasc. Dis. Women WINCARS 3 (4) (2018) 240–244.
[43] H. Abdollahi, M. Abdolahi, M. Sedighiyan, A. Jafarieh, The effect of L-carnitine on mortality rate in septic patients: a systematic review and meta-analysis on randomized clinical trials, Endocr., Metab. Immune Disord.: Drug Targets 21 (4) (2021) 673–681.
[44] M.F.N. Meeran, S. Azimullah, E. Adeghate, S. Ojha, Nootkatone attenuates myocardial oxidative damage, inflammation, and apoptosis in isoproterenol-induced myocardial infarction in rats, Phytomed 84 (2021) 153405.
[45] C. Hu, X. Zhang, N. Zhang, W.-Y. Wei, L.-L. Li, Z.-G. Ma, Q.-Z. Tang, Osteocrin attenuates inflammation, oxidative stress, apoptosis, and cardiac dysfunction in doxorubicin-induced cardiotoxicity, Clin. Transl. Med. 10 (3) (2020) e124.
[46] J. Ye, Y. Huang, B. Que, C. Chang, W. Liu, H. Hu, L. Liu, Y. Shi, Y. Wang, M. Wang, T. Zeng, W. Zhen, Y. Xu, L. Shi, J. Liu, H. Jiang, D. Ye, Y. Lin, J. Wan, Q. Ji, Interleukin-12p35 knock out aggravates doxorubicin-induced cardiac injury and dysfunction by aggravating the inflammatory response, oxidative stress, apoptosis and autophagy in mice, EBioMedicine 35 (2018) 29–39.
[47] P.M. Kang, S. Izumo, Apoptosis in heart: basic mechanisms and implications in cardiovascular diseases, Trends Mol. Med. 9 (4) (2003) 177–182.
[48] A. Haunstetter, S. Izumo, Toward antiapoptosis as a new treatment modality, Circ. Res. 86 (4) (2000) 371–376.
[49] H.H. Mansour, S.M. El kiki, A.B. Ibrahim, M.M. Omran, Effect of L-carnitine on cardiotoxicity and apoptosis induced by imatinib through PDGF/PPARγ/MAPK pathways, Arch. Biochem. Biophys. 704 (2021) 108866.
[50] M.A. Savitskaya, G.E. Onishchenko, Mechanisms of apoptosis, Biochem. Mosc. 80 (11) (2015) 1393–1405.
[51] Y.-L. Zhang, G.-Y. Li, Y.-Y. Zhao, X.-Y. Dai, M.-W. Hu, H.-B. Cao, K. Huang, F. Yang, Inhibition of calcium imbalance protects hepatocytes from vanadium exposure-induced inflammation by mediating mitochondrial-associated endoplasmic reticulum membranes in ducks, Poult. Sci. 102 (12) (2023) 103013.
[52] X.-L. Dong, C.-P. Li, Y.-Y. Yao, F.-Z. Liu, P. Jiang, Y. Gao, Xingnaojing injection alleviates cerebral ischemia/reperfusion injury through regulating endoplasmic reticulum stress in Vivo and in Vitro, Heliyon 10 (3) (2024) e25267.
[53] Q. Wang, W.-Y. Chen, B.-Y. Zhang, Z. Gao, Q.-P. Zhang, H.-Q. Deng, L.-Y. Han, X.-L. Shen, Perfluorooctanoic acid induces hepatocellular endoplasmic reticulum stress and mitochondrial-mediated apoptosis in vitro via endoplasmic reticulum-mitochondria communication, Chem. Biol. Interact. 354 (2022) 109844.
[54] Y. Zhou, D.D. Murugan, H. Khan, Y. Huang, W.S. Cheang, Roles and therapeutic implications of endoplasmic reticulum stress and oxidative stress in cardiovascular diseases, Antioxidants 10 (8) (2021) 1167.
[55] J. Ye, Y. Han, X. Chen, J. Xie, X. Liu, S. Qiao, C. Wang, L-carnitine attenuates H_2O_2-induced neuron apoptosis via inhibition of endoplasmic reticulum stress, Neurochem. Int. 78 (2014) 86–95.
[56] D.G. Breckenridge, M. Germain, J.P. Mathai, M. Nguyen, G.C. Shore, Regulation of apoptosis by endoplasmic reticulum pathways, Oncogene 22 (53) (2003) 8608–8618.
[57] H. Li, W. Pan, C. Li, M. Cai, W. Shi, Z. Ren, H. Lu, Q. Zhou, H. Shen, Heat stress induces calcium dyshomeostasis to subsequent cognitive impairment through ERS-mediated apoptosis via SERCA/PERK/eIF2α pathway, Cell Death Discov 10 (1) (2024) 280.
[58] Y. Yang, C.-W. Li, N. Liu, M.-M. Wang, X.-M. Zhou, I.-H. Kim, Z.-L. Wu, Ursolic acid alleviates heat stress-induced lung injury by regulating endoplasmic reticulum stress signaling in mice, J. Nutr. Biochem. 89 (2021) 108557.
[59] Y.-G. Lee, H.-C. Chou, Y.-T. Chen, S.-Y. Tung, T.-L. Ko, B. Buyandelger, L.-L. Wen, S.-H. Juan, L-carnitine reduces reactive oxygen species/endoplasmic reticulum stress and maintains mitochondrial function during autophagy-mediated cell apoptosis in perfluorooctanesulfonate-treated renal tubular cells, Sci. Rep. 12 (1) (2022) 4673.