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Trehalose-Induced Activation of Autophagy Improves Cardiac Remodeling After Myocardial Infarction

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ABSTRACT

BACKGROUND Trehalose (TRE) is a natural, nonreducing disaccharide synthesized by lower organisms. TRE exhibits an extraordinary ability to protect cells against different kinds of stresses through activation of autophagy. However, the effect of TRE on the heart during stress has never been tested.

OBJECTIVES This study evaluated the effects of TRE administration in a mouse model of chronic ischemic remodeling.

METHODS Wild-type (WT) or *beclin 1*+/− mice were subjected to permanent ligation of the left anterior descending artery (LAD) and then treated with either placebo or trehalose (1 mg/g/day intraperitoneally for 48 h, then 2% in the drinking water). After 4 weeks, echocardiographic, hemodynamic, gravimetric, histological, and biochemical analyses were conducted.

RESULTS TRE reduced left ventricular (LV) dilation and increased ventricular function in mice with LAD ligation compared with placebo. Sucrose, another nonreducing disaccharide, did not exert protective effects during post-infarction LV remodeling. Trehalose administration to mice overexpressing GFP-tagged LC3 significantly increased the number of GFP-LC3 dots, both in the presence and absence of chloroquine administration. TRE also increased cardiac LC3-II levels after 4 weeks following myocardial infarction (MI), indicating that it induced autophagy in the heart in vivo. To evaluate whether TRE exerted beneficial effects through activation of autophagy, trehalose was administered to *beclin 1*+/− mice. The improvement of LV function, lung congestion, cardiac remodeling, apoptosis, and fibrosis following TRE treatment observed in WT mice were all significantly blunted in *beclin 1*+/− mice.

CONCLUSIONS TRE reduced MI-induced cardiac remodeling and dysfunction through activation of autophagy. (J Am Coll Cardiol 2018;71:1999–2010) © 2018 by the American College of Cardiology Foundation.

Cardiovascular diseases remain the greatest cause of death in Western countries (1). The final common pathway of chronic cardiovascular disorders, including coronary artery disease, is heart failure, which is a highly morbid and disabling condition that can lead to death after recurrent hospitalizations (1,2). Therefore, it is important to develop pharmacological therapies that target chronic cardiac remodeling following myocardial injury (e.g., acute myocardial infarction [MI]) to reduce the incidence of heart failure and death.

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ABBREVIATIONS AND ACRONYMS

***beclin 1*+/−** = heterozygous *Beclin*-1 knock-out

ER = endoplasmic reticulum

LAD = left anterior descending coronary artery

LV = left ventricular

LVEDD = left ventricular end-diastolic diameter

LVESD = left ventricular end-systolic diameter

SUC = sucrose

TFEB = transcription factor EB

Tg-GFP-LC3 mice = transgenic mice expressing LC3 conjugated with a green fluorescent protein

TRE = trehalose

WT = wild type

We hypothesized that trehalose (TRE) might be a potentially beneficial compound for the treatment of chronic ischemic remodeling. TRE is a natural, nonreducing α-linked disaccharide composed of 2 molecules of glucose that is synthesized by lower organisms, such as yeasts, bacteria, insects, and plants; however, it is not synthesized by mammals (3-6). Accumulating lines of evidence indicate that TRE has an extraordinary ability to protect cells in response to different kinds of stresses (3-6). TRE rapidly accumulates in lower organisms such as yeasts and tardigrades, enabling them to survive dehydration, thermal shock, oxidative stress, and protein aggregation (3-6). TRE can also protect mammalian cells against stress. TRE confers a high water retention capability to cells, thereby protecting intracellular organelles from disruption by hydration/dehydration cycles during stress (7,8). In addition, TRE elicits antioxidant functions (5,9,10) and dramatically reduces intracellular protein aggregates and misfolded protein accumulation (11). It exerts salutary effects in mouse models of neurodegenerative disorders by promoting the clearance of β-amyloids and huntingtin aggregates (12-14). In addition, TRE can reduce hepatic steatosis by promoting the clearance of intracellular lipid droplets (15). However, the effect of TRE administration during cardiac stress is currently unknown.

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The clearance of protein aggregates by TRE is accompanied by induction of autophagy in COS-7 and PC12 cells (11). Autophagy is an evolutionarily conserved intracellular mechanism that mediates degradation of misfolded proteins, lipid aggregates, and damaged organelles (16). Autophagy is activated in response to cellular stresses, including starvation, endoplasmic reticulum (ER) stress, and oxidative stress, thereby limiting cell death (16). Genetic or pharmacological inhibition of autophagy exacerbates myocardial ischemic injury and chronic cardiac remodeling in mouse models of myocardial ischemia and MI (17-20). Conversely, activation of autophagy limits myocardial damage in response to ischemia, and reduces chronic ischemic remodeling and heart failure (18,21,22). The salutary effects of autophagy are mediated by the preservation of energy content, the clearance of misfolded proteins and/or damaged intracellular organelles, and the improvement of mitochondrial function. However, clinical application of interventions to stimulate autophagy is challenging due to issues of specificity and the side effects inherent to each intervention. Thus, the pharmacological compound most suitable for activating autophagy has yet to be identified (23).

The aim of this study was to test whether TRE administration reduces cardiac remodeling and dysfunction in a mouse model of chronic MI, and, if so, whether these effects are mediated by autophagy activation.

METHODS

ANIMAL MODELS AND TRE ADMINISTRATION. Heterozygous transgenic mice expressing LC3 conjugated with a green fluorescent protein (Tg-GFP-LC3) (C57BL/6J background, strain GFP-LC3#53, RIKEN BioResource Center, Tsukuba, Japan) containing a rat LC3-EGFP fusion under control of the chicken β-actin promoter and heterozygous Beclin-1 knock-out (*beclin 1*+/−) mice (C57BL/6J background) were bred in-house, as previously described (21).

Wild-type (WT) C57BL/6J mice were subjected to chronic MI for 4 weeks by permanent left anterior descending coronary artery (LAD) ligation. After LAD ligation, mice were divided into 3 treatment groups: 1 group was treated with TRE (1 mg/g/day intraperitoneally for 48 h, then 2% in the drinking water until the end of the 4-week period), whereas the other 2 groups were control groups that received either placebo (saline for the initial 48 h and then regular water) or sucrose (SUC) (same dosage as TRE). Sham mouse groups that were not subjected to LAD ligation also received placebo, SUC, or TRE. After 4 weeks, echocardiographic, hemodynamic, gravimetric, histological, and biochemical analyses were conducted as previously described (21,24).

In a different set of experiments, WT and *beclin 1*+/− mice were subjected to LAD ligation and received either placebo or TRE for 4 weeks. Finally, to evaluate the acute effects of TRE on myocardial autophagy and autophagic flux, Tg-GFP-LC3 mice were administered either TRE (1 mg/g/day intraperitoneally) or placebo (saline) for 48 h, with or without chloroquine administration (10 mg/kg intraperitoneally 4 h before being killed), as previously described (21,25). TRE, chloroquine, and SUC were all purchased from Sigma-Aldrich (St. Louis, Missouri). All animal protocols were approved by the local Institutional Animal Care and Use Committee of Rutgers New Jersey Medical School.

LAD LIGATION PROCEDURES. Mice were anesthetized by intraperitoneal administration of pentobarbital sodium (60 mg/kg), and then ventilated for the entire procedure through an endotracheal tube connected to a mouse ventilator. The LAD was visualized through a left thoracotomy across the third intercostal space, and then ligated 1 to 2 mm distal to the left atrial appendage with an 8-0 nylon suture placed around the artery. After closure of the chest wall and extubation, mice were placed in a recovery cage with the temperature maintained at 37°C overnight and then housed normally.

ECHOCARDIOGRAPHIC ANALYSIS. Mice were anesthetized by intraperitoneal injection of 2, 2, 2-tribromoethanol (300 mg/kg, Sigma-Aldrich). Mouse chests were shaved, and the animals were positioned on a warm cushion. All left ventricular (LV) measurements were taken in the M-mode short-axis view at the level of papillary muscles. Left ventricular end-diastolic diameter (LVEDD) and diastolic wall measurements were obtained at the time of the apparent maximal diastolic diameter, whereas left ventricular end-systolic diameter (LVESD) and systolic wall measurements were obtained at the time of the most anterior systolic excursion of the posterior wall. LV fractional shortening was calculated as follows: fractional shortening = (LVEDD − LVESD)/LVEDD × 100.

HEMODYNAMIC ANALYSIS. Pressure−volume analysis was performed using the Millar PV system MPVS-300/400 (Millar Instruments, Houston, Texas). After anesthesia with 2, 2, 2-tribromoethanol (300 mg/kg; Sigma-Aldrich), the right carotid artery was cannulated with a high-fidelity Mikro-Tip catheter transducer (1.0-F, Model PVR-1030, Millar Instruments). LV diastolic and systolic pressures and performance were measured as previously described (26,27).

HISTOLOGICAL ANALYSES. De-paraffinized tissue sections were antigen-unmasked, and wheat germ agglutinin staining and Masson's trichrome staining were performed as previously described (21,24). For GFP-LC3 dot visualization in Tg-GFP-LC3 mice, myocardial samples were embedded in Tissue-Tek OCT compound (Sakura Finetechnical Co. Ltd., Tokyo, Japan) and stored at −80°C. The samples were sectioned at 5- to 7-µm thickness with a cryostat (CM3050 S, Leica Biosystems, Buffalo Grove, Illinois). GFP-LC3 dots were observed under a fluorescence microscope as previously described (17,21).

For immunofluorescent staining, fixed cardiomyocytes were incubated overnight with anti-transcription factor EB (TFEB) antibody (MyBioSource, San Diego, California) and then with Alexa Fluor 568 dye-conjugated secondary antibody (Life Technologies, Carlsbad, California). Samples were washed and mounted on glass slides with 4′,6-diamidino-2-phenylindole (DAPI) (Vectashield, Vector Laboratories, Burlingame, California).

CELL CULTURES. Neonatal rat cardiomyocytes were isolated and cultured as previously reported (21). Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. To knock down TFEB, cardiomyocytes were infected with an adenovirus that expressed a short hairpin sequence targeting TFEB for 72 h. To evaluate the effects of TRE on mitophagy, cardiomyocytes were transduced with an adenovirus that harbored mitochondria-targeted keima, a protein that emits different fluorescent signals depending on the microenvironment pH. In this way, it was possible to track the localization of mitochondria inside lysosomes (acidic microenvironment). Mitophagy was assessed as previously described (28).

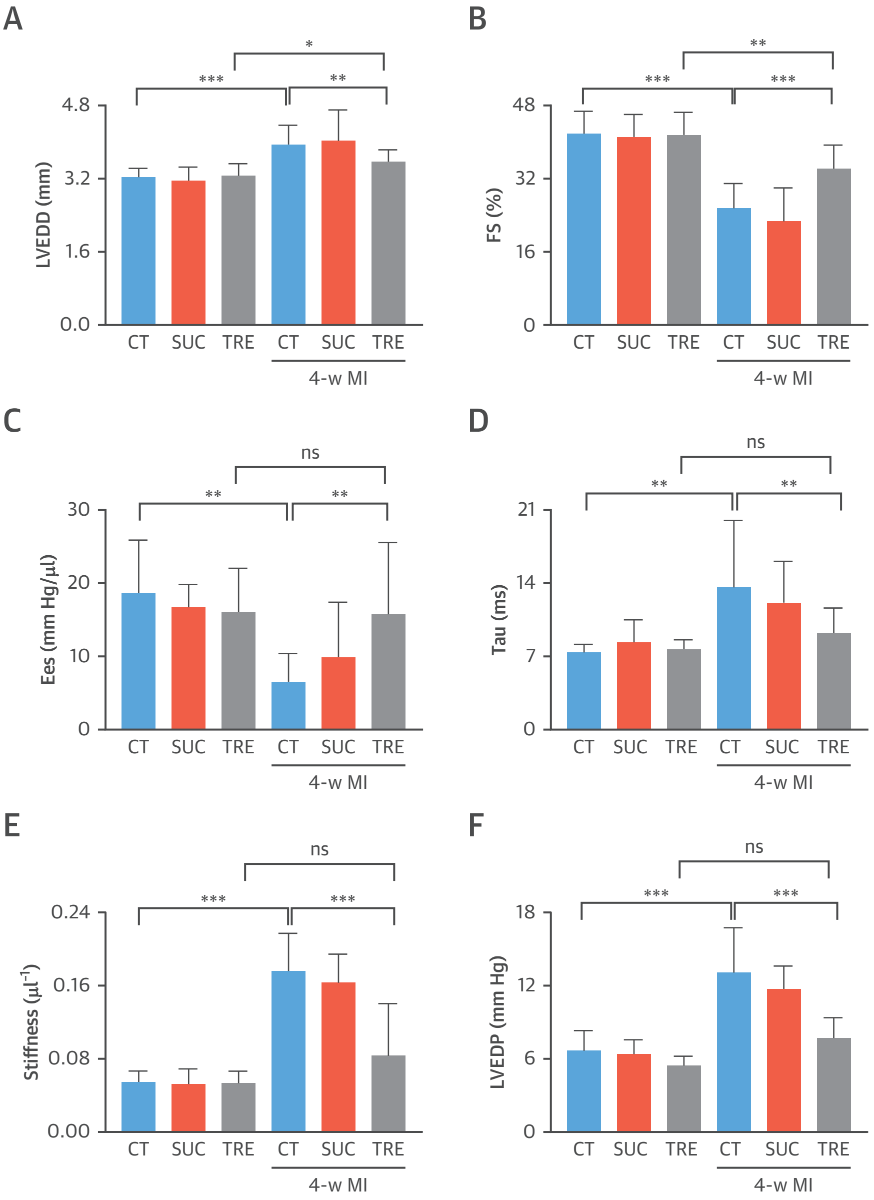
IMMUNOBLOT ANALYSIS AND ANTIBODY. Myocardial and cardiomyocyte samples were lysed in radioimmunoprecipitation assay (RIPA) buffer, and immunoblot analyses were performed as previously described (24). Nuclear and cytosolic fractions were isolated from LV samples as previously described (29). Anti-LC3 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibodies were purchased from MBL International (Woburn, Massachusetts) and Cell Signaling Technology, Inc. (Danvers, Massachusetts), respectively. Antibodies detecting cleaved Caspase-3, Histone H3, p-ERK1/2 (Thr202/Tyr204), and total ERK1/2 were purchased from Cell Signaling Technology, Inc. Ubiquitin antibody was purchased from Santa Cruz Biotechnology, Inc. (Dallas, Texas). Anti-SERCA2 and anti-Cathepsin D antibodies were purchased from Novus Biologicals (Littleton, Colorado) and Abcam (Cambridge, Massachusetts) respectively.

STATISTICAL ANALYSIS. Continuous variables were expressed as mean ± SD. The Student's *t*-test was used to compare means of 2 groups. When ≥3 groups were specifically intercompared, the 1-way analysis of variance test was used, followed by the Bonferroni post hoc test. A p value <0.05 was considered statistically significant. Analyses were performed using Graphpad 7 and SPSS statistical software, version 20 (IBM, Armonk, New York).

RESULTS

TRE ATTENUATES CHRONIC ISCHEMIC REMODELING. To evaluate the effects of TRE on cardiac remodeling after MI, WT C57BL/6J mice were subjected to LAD ligation and treated with either placebo, TRE, or SUC, as depicted in Online Figure 1. After 4 weeks, echocardiographic analyses showed that mice with LAD ligation treated with placebo displayed significant LV dilation and a reduction in systolic LV function compared with control mice that underwent a sham operation and placebo treatment. In contrast, mice with LAD ligation treated with TRE exhibited attenuated LV remodeling compared with those with LAD

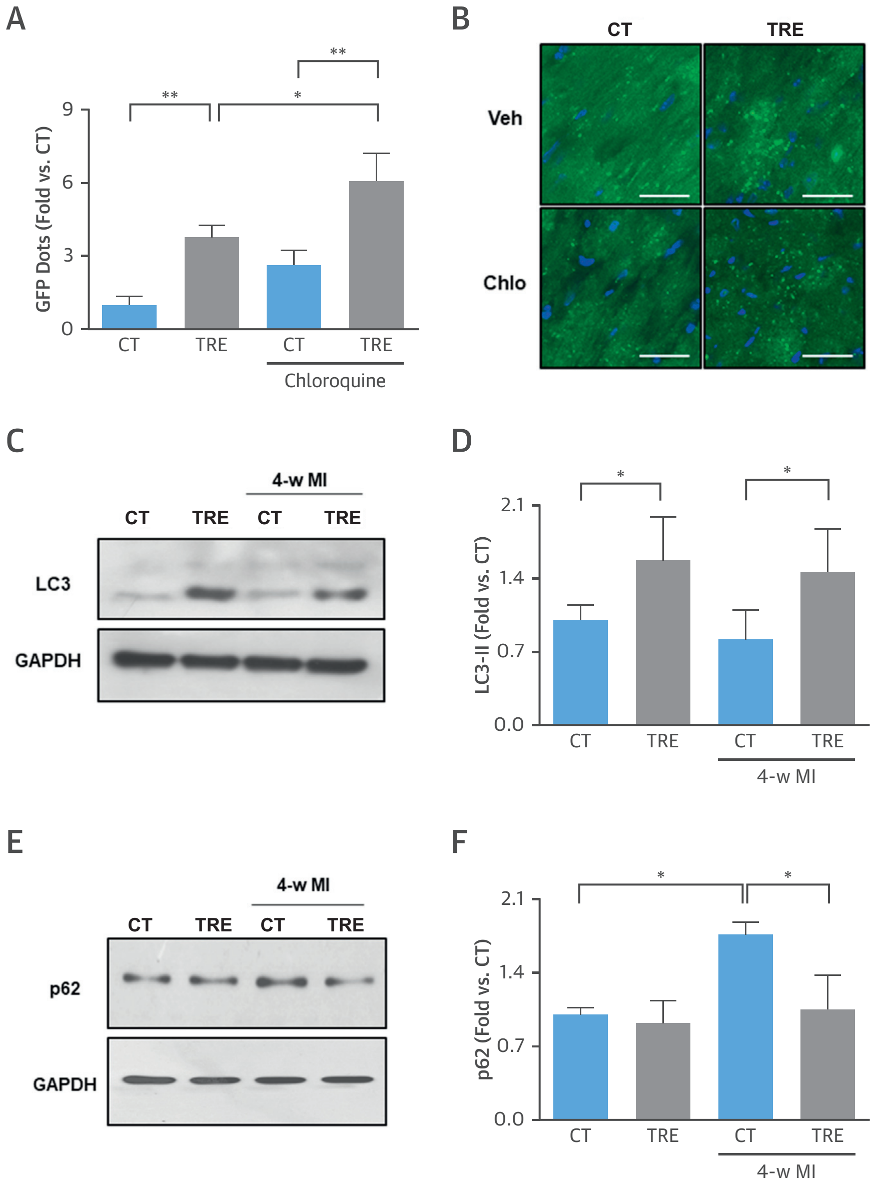
FIGURE 1 **Trehalose Treatment Ameliorates Chronic Ischemic Remodeling**



(A to F) Mice with and without left anterior descending artery (LAD) ligation received placebo (CT), sucrose (SUC), or trehalose (TRE). After 4 weeks, they were subjected to echocardiographic analysis to assess **(A)** left ventricular end-diastolic diameter (LVEDD) and **(B)** fractional shortening (FS). n = 9 in placebo sham group; n = 9 in SUC sham group; n = 10 in TRE sham group; n = 19 in placebo myocardial infarction (MI) group; n = 14 in SUC MI group; n = 21 in TRE MI group. Mice were also subjected to hemodynamic analysis to assess the slope of the **(C)** end-systolic pressure volume relationship (ESPVR), **(D)** the relaxation constant Tau, **(E)** the left ventricular stiffness, and **(F)** the left ventricular end-diastolic pressure (LVEDP). n = 7 in each sham group; n = 18 in placebo MI group; n = 14 in SUC MI group; n = 19 in TRE MI group. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Ees = end-systolic elastance; ns = not significant; w = weeks.

ligation treated with placebo and with sham-operated groups (**Figures 1A and 1B,** Online Figure 2A). Interestingly, SUC treatment did not exert any protective effect with respect to LV remodeling after LAD ligation, and its effects were comparable to those of placebo (**Figures 1A and 1B,** Online Figure 2A). Hemodynamic studies also showed that TRE treatment significantly ameliorated systolic and diastolic dysfunction after 4 weeks of MI, as indicated by the significantly increased slope of the end-systolic

FIGURE 2 **Trehalose Promotes Myocardial Autophagy**



(A and B) Transgenic mice expressing LC3 conjugated with a green fluorescent protein (Tg-GFP-LC3) received placebo (saline) or TRE (1 mg/g/day intraperitoneally) for 48 h. In some animals, chloroquine was also administered (10 mg/kg intraperitoneally, 4 h before being killed). **(A)** Quantification of the number of GFP-LC3 dots per field is shown together with representative pictures of myocardial dots. **(B)** Quantification of the number of dots per field. Data are expressed as fold versus placebo without chloroquine (CT). n = 4 per group. Scale bar: 50 μm. **(C to F)** Mice with LAD ligation received CT or TRE. After 4 weeks, cardiac levels of LC3, p62, and GAPDH were analyzed by immunoblot. **(C and E)** A representative immunoblot is shown, together with the **(D)** densitometric analysis of normalized LC3-II and p62 levels. Data are expressed as fold versus CT. n = 4 to 5 per group. $^*p < 0.05$; $^{**}p < 0.01$. Chlo = chloroquine; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; GFP = green fluorescent protein; Veh = vehicle; other abbreviations as in Figure 1.

pressure volume relationship, lower LV relaxation constant (Tau), and lower index of myocardial stiffness in the TRE-treated group compared with the placebo group (Figures 1C to 1E). LV end-diastolic pressure was significantly lower in the TRE-treated group than in the placebo group (Figure 1F). TRE-treated groups with MI also did not exhibit a significant alteration of these hemodynamic parameters with respect to sham-operated groups. Taken together, these results indicated that TRE reduced LV remodeling and improved both systolic and diastolic function in mice with chronic MI. These effects were specific to TRE and not to disaccharides in general because SUC did not exhibit similar beneficial effects on ischemic remodeling.

TRE INDUCES AUTOPHAGY IN THE HEART. Previous studies demonstrated that TRE exerted beneficial effects in other organs, in part through activation of autophagy (11,14,15,30). To test whether TRE induced autophagy in the heart, we administered TRE to Tg-GFP-LC3 mice and evaluated the extent of autophagosome formation. We found that 48 h of TRE treatment significantly increased the number of LC3 puncta in both the absence and presence of chloroquine, a lysosome inhibitor (**Figures 2A and 2B**) (25). This result indicated that TRE promoted both autophagosome formation and autophagic flux in the heart. Long-term treatment with TRE also increased autophagy both in control mice without MI and in mice subjected to 4 weeks of LAD ligation, as indicated by an increase in LC3-II, which is a biochemical indicator of autophagosome accumulation (**Figures 2C and 2D**). TRE treatment also significantly attenuated the accumulation of p62, a protein that is degraded by autophagy, in the hearts of mice with chronic MI, which further demonstrated that this molecule induces cardiac autophagy (**Figures 2E and F**). In addition, long-term TRE treatment enhanced autophagosome formation in the hearts of mice with LAD ligation, as indicated by the increased number of LC3 puncta in Tg-GFP-LC3 mice treated with TRE compared with those treated with placebo, either with or without chloroquine treatment (Online Figure 3). Furthermore, we observed an increase in the number of LC3 dots in the hearts of Tg-GFP-LC3 mice treated with TRE in response to chloroquine administration, but this was not seen in placebo-treated mice. This suggested a reduction of autophagic flux in the heart during the chronic phase of cardiac remodeling that could be recovered by TRE administration, which was consistent with the observed p62 accumulation (**Figures 2E and 2F**) and with previously published evidence (31). Finally, we found that myocardial levels of cathepsin D were increased by TRE treatment, which suggested enhanced lysosomal biogenesis, which might have contributed to the observed increase in autophagic flux (Online Figure 4).

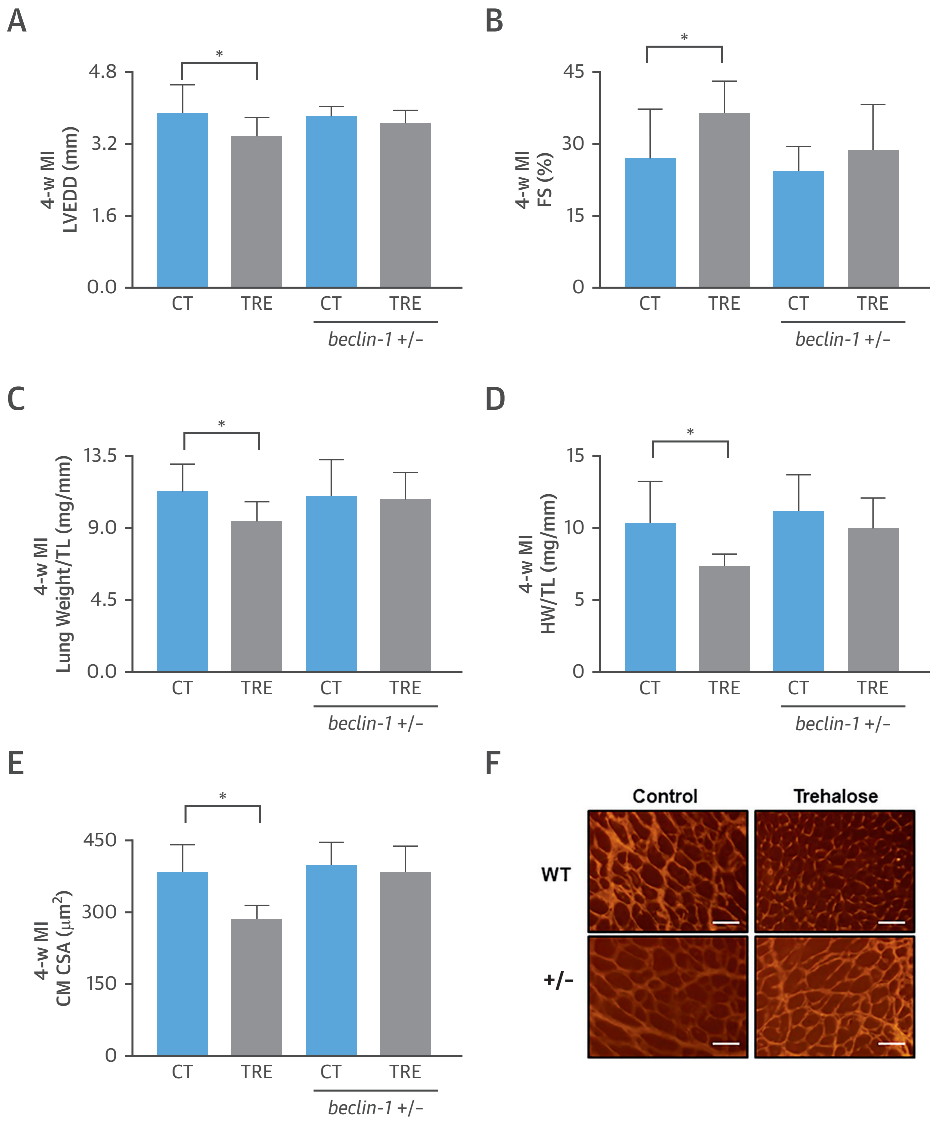
Previous work demonstrated that autophagy activation limits cardiomyocyte apoptosis, misfolded protein accumulation, and mitochondrial dysfunction in response to stress (17–22). In line with this evidence, we found reductions in cleaved caspase 3 levels and protein ubiquitination in mice with chronic MI treated with TRE (Online Figures 5A and 5B). TRE treatment also increased SERCA2 levels in mice with LAD ligation (Online Figure 5A), in accordance with the increased cardiac contractility observed in these animals.

Then, we tested whether TRE promoted mitophagy in cardiomyocytes. Mitophagy is a critical mechanism for degradation of damaged mitochondria and preservation of mitochondrial function. TRE promoted mitophagy in cardiomyocytes at baseline and in response to energy deprivation, as indicated by an increased number of keima dots with a high 560/440 fluorescent signal ratio, which corresponded to the mitochondria localized in lysosomes (Online Figure 6).

TRE INDUCES AUTOPHAGY THROUGH UP-REGULATION OF TFEB. It is known that TRE induces autophagy independently of the mTOR pathway in COS-7 cells (11). Previous studies showed that TRE promoted activation of TFEB (32–34), which is involved in up-regulation of autophagy genes and activation of autophagy (35,36). We found that TRE promoted nuclear localization of TFEB in cardiomyocytes in vitro and in the mouse heart in vivo, which confirmed that TFEB was activated (Online Figures 7A and 7B). Importantly, we found that TFEB knockdown significantly attenuated TRE-induced cardiomyocyte autophagy and survival in response to hydrogen peroxide (Online Figures 7C to 7E), which suggested that TFEB mediated the beneficial effects of TRE in cardiomyocytes. Bafilomycin treatment also attenuated the antiapoptotic effect of TRE in cardiomyocytes exposed to hydrogen peroxide (Online Figure 7F), which indicated that preserved lysosomal function is required for the beneficial effects of TRE in response to stress in cardiomyocytes and is likely to maintain autophagic flux. Of note, it was previously demonstrated that TFEB nuclear localization might be mediated by inhibition of the ERK signaling pathway (35). However, TRE administration did not significantly modulate ERK activity as evaluated by its phosphorylation in the mouse heart either at baseline or during chronic MI (Online Figures 8A and 8B), which suggested that this pathway might not be important for TRE-induced TFEB nuclear localization.

TRE ATTENUATES CHRONIC ISCHEMIC REMODELING THROUGH THE ACTIVATION OF AUTOPHAGY. To evaluate whether TRE attenuated ischemic remodeling through the activation of autophagy, WT and

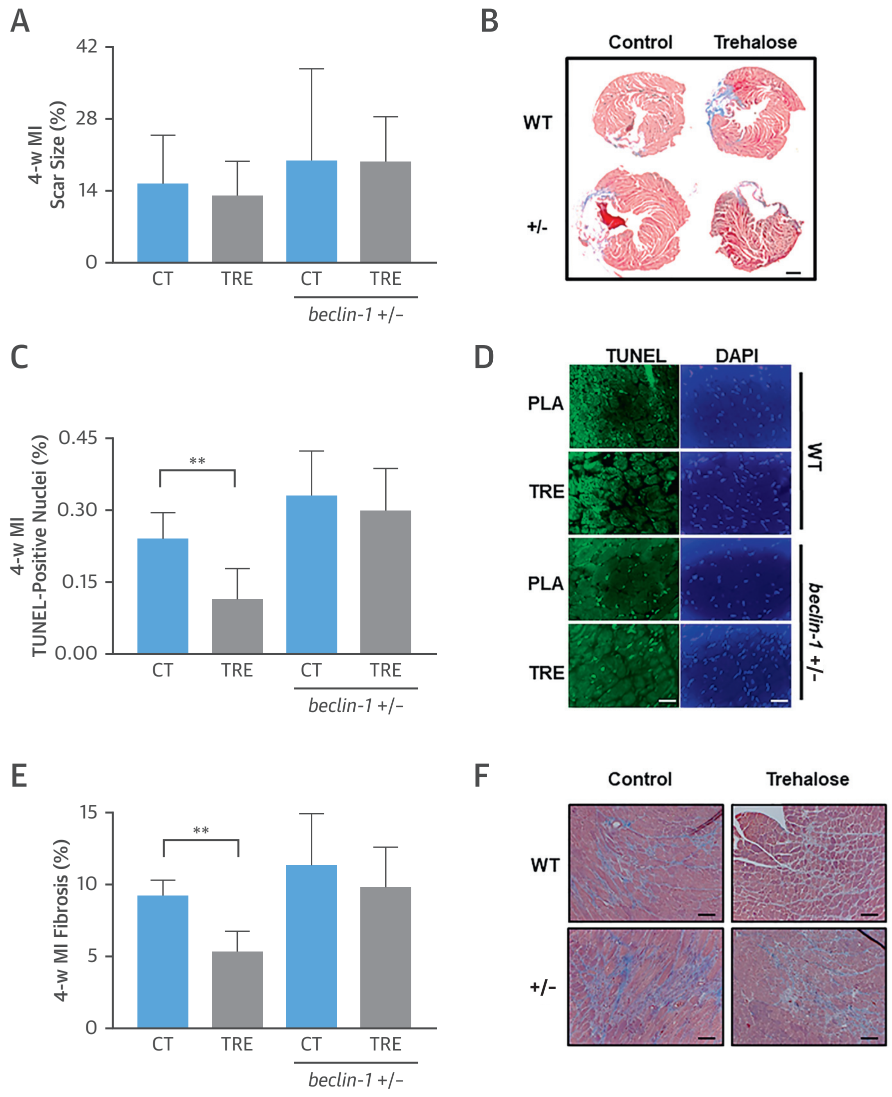
FIGURE 3 **Beneficial Effects of Trehalose on Cardiac Remodeling and Function in Response to LAD Ligation Are Attenuated in *beclin 1*+/− Mice**



(A to F) Wild-type (WT) and *beclin 1*+/− mice with LAD ligation received CT or TRE. After 4 weeks, they were subjected to echocardiographic analysis to assess **(A)** LVEDD and **(B)** FS. **(C)** Lung and **(D)** heart weight were also measured. n = 9 in placebo group; n = 11 in TRE group; n = 8 in *beclin-1* +/− placebo group; n = 9 in *beclin-1* +/− TRE group. Cell size was measured in left ventricular myocardial tissue sections. Quantification of **(E)** the cross-sectional area (CSA), together with **(F)** representative pictures of wheat germ agglutinin staining are shown. Data are presented as fold versus WT CT. n = 4 per group. *$p < 0.05$. Scale bar: 50 μm. HW = heart weight; TL = tibia length; other abbreviations as in Figure 1.

beclin 1+/− mice were subjected to LAD ligation and received either placebo or TRE treatment for 4 weeks. Autophagy is genetically disrupted in *beclin 1*+/− mice, and these mice are insensitive to autophagy inducers (21,28). Although TRE significantly attenuated LAD ligation-induced LV dilation and systolic dysfunction in WT mice, these effects were blunted in *beclin 1*+/− mice (Figures 3A and 3B). Similarly, TRE significantly reduced lung weight, a sign of lung congestion and heart failure, in WT mice, but not in *beclin 1*+/− mice subjected to chronic MI (Figure 3C). Heart weight normalized for tibial length and

FIGURE 4 Beneficial Effects of Trehalose on Cardiac Apoptosis and Fibrosis in Response to LAD Ligation Are Attenuated in *beclin 1*+/− Mice

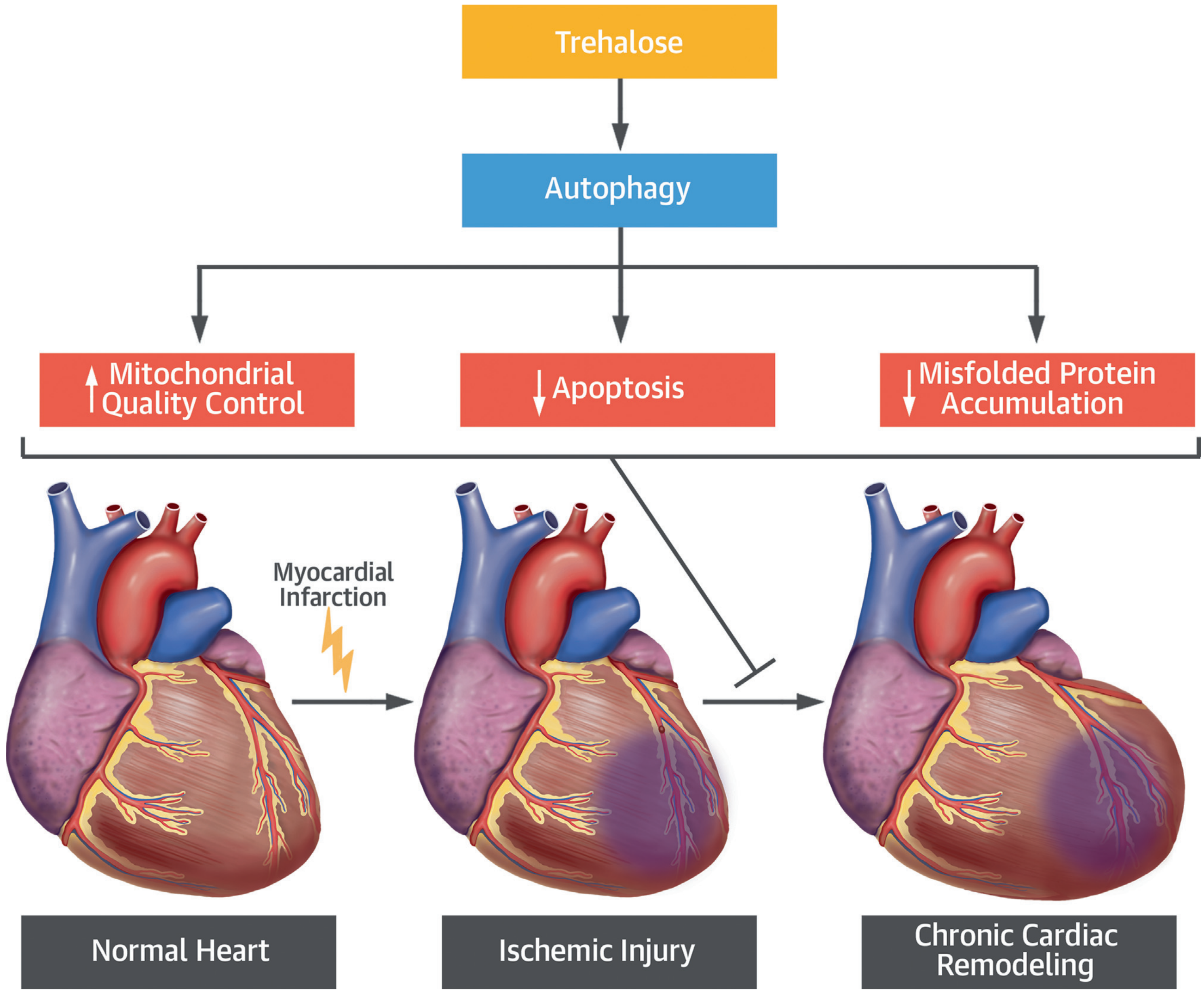


(A to F) WT and *beclin 1*+/− mice with LAD ligation received CT or TRE. **(A)** After 4 weeks, infarct size was measured. **(B)** Representative pictures of Masson's trichrome staining of the whole left ventricular transverse section are also shown. Scale bar: 1 mm. **(C and D)** Apoptosis was evaluated by quantification of TUNEL-positive cells. Data are expressed as fold versus CT. n = 4 to 7 per group. **(E)** The percentage of cardiac fibrosis was also quantified, and **(F)** representative pictures of Masson's trichrome staining are shown. n = 4 per group. $^{**}p < 0.01$. Scale bar: 50 µm. DAPI = 4′,6-diamidino-2-phenylindole; PLA = placebo; TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling; other abbreviations as in Figure 1.

cardiomyocyte size after 4 weeks of MI were also reduced by TRE in WT mice, but these effects were blunted in *beclin 1*+/− mice (Figures 3D to 3F).

TRE treatment did not significantly affect the MI scar size 4 weeks after LAD ligation in either WT or *beclin 1*+/− mice, as evaluated with Masson's trichrome staining (Figures 4A and 4B). However, cardiomyocyte apoptosis in the remote area was significantly reduced by TRE treatment in WT mice but not in *beclin 1*+/− mice (Figures 4C and 4D). TRE treatment also significantly reduced myocardial fibrosis in WT mice after 4 weeks of MI compared with placebo treatment, whereas it failed to reduce myocardial fibrosis in *beclin 1*+/− mice (Figures 4E and 4F). These results demonstrated that TRE treatment reduced cardiac remodeling and dysfunction, cardiac

CENTRAL ILLUSTRATION **Trehalose Administration Hampers the Detrimental Cellular Effects Induced by Chronic Myocardial Infarction and Reduces Cardiac Remodeling by Activating Autophagy**



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Autophagy activation by trehalose increases mitochondrial quality control and attenuates misfolded protein accumulation and apoptosis induced by chronic myocardial infarction.

hypertrophy, apoptosis, and fibrosis in response to chronic MI, at least partially through activation of autophagy (Online Figure 9).

DISCUSSION

In the present study, we investigated the effects of TRE administration on LV remodeling after chronic MI. Long-term TRE treatment significantly attenuated MI-induced cardiac remodeling and improved both systolic and diastolic LV function (**Central Illustration**). These beneficial effects were associated with a reduction in cardiac hypertrophy, apoptosis, and fibrosis. TRE significantly activated autophagy in the heart, and the cardioprotective effect of TRE during cardiac remodeling was blunted in mice with a genetic disruption of autophagy, which suggested that autophagy activation mediates the salutary effects of TRE. Because SUC, another nonreducing disaccharide, did not exhibit the same beneficial effects on cardiac remodeling that TRE did, the protective effects of TRE would appear to be specific.

Our work significantly extended previous evidence that demonstrated that TRE protects cells in response to stress. In lower organisms, TRE was rapidly synthesized in response to stress, thereby preserving cell viability and functions (3-6). In contrast, TRE was not synthesized in mammals. However, several studies demonstrated that exogenously applied TRE exerted beneficial effects on mammalian cells in response to oxidative stress, DNA damage, and misfolded protein accumulation (9,11,37). TRE was also shown to ameliorate pathological conditions in animal models of human diseases in vivo. For example, long-term oral administration of TRE to a mouse model of Huntington disease dramatically ameliorated motor dysfunction, extended survival, and reduced polyglutamine aggregates (12). Long-term treatment with TRE, but not SUC, also preserved motor neuron survival and mitochondrial function in a mouse model of amyotrophic lateral sclerosis, thereby delaying the onset of the disease and extending the lifespan (14). TRE reduced intracellular protein aggregates and reduced neuron death in a model of Alzheimer's disease (13). Recently, TRE administration in drinking water was found to reduce high fructose–induced hepatic steatosis through increased clearance of intracellular lipid droplets (15).

Previous studies suggested that the beneficial effects of TRE might be mediated by autophagy activation (11,13-15). In this study, for the first time, we provided in vivo genetic evidence that the protective effects of TRE on cardiac remodeling were mediated through stimulation of autophagy because these effects were lost when TRE was administered to *beclin 1*+/− mice.

The mechanisms through which TRE induces autophagy remain to be clarified. It has been suggested that this process is independent of the mTOR pathway (11). We found that TRE induced dramatic nuclear localization of TFEB and that TFEB knockdown attenuated the pro-autophagic and pro-survival effects exerted by TRE. These data suggested that TRE promotes autophagy in part through stimulation of TFEB, which is in line with previous evidence (32-34). TFEB promoted not only lysosomal biogenesis, but also autophagosome formation by directly controlling the expression of autophagy-related genes (35,36). In addition, like TRE, TFEB activation dramatically reduced protein aggregates (38). Future studies are warranted to understand how TRE regulates TFEB. The Akt and AMPK signaling pathways were previously shown to be involved in these mechanisms (15,34). In addition, it will be important to conduct studies to evaluate whether TFEB is required for TRE-induced autophagy in vivo and to clarify the specific role of lysosomal biogenesis in the beneficial effects of TRE.

We showed previously that autophagy is negatively regulated by Mst1, a pro-apoptotic kinase, through Thr108 phosphorylation of Beclin1, during cardiac remodeling after MI (21). Cardiac remodeling and LV dysfunction are often accompanied by oxidative stress and calcium overload, and consequent development of mitochondrial dysfunction and ER stress (39). Accumulation of protein aggregates and dysfunctional mitochondria and ER is often observed in the failing heart (39). Because autophagy protects the heart not only through recycling of adenosine triphosphate but also by eliminating protein aggregates and damaged intracellular organelles (e.g., mitochondria), an intervention that stimulates autophagy should be beneficial. Previous studies showed that interventions to stimulate autophagy, including rapamycin (40), inhibition of Mst1 (21), and TAT-Beclin 1 (28), improved the function of the heart in the presence of hemodynamic overload. Considering the specificity and potential toxicity of these available interventions in experimental animals, the development of safe and effective interventions to stimulate autophagy is needed.

Our study suggested that TRE might represent a potentially useful molecule to reduce cardiac remodeling and heart failure in human patients, and to activate autophagy in a physiological manner. TRE is a natural compound with apparently no side effects in human subjects. It can be found in certain foods, and it is currently used as a sweetener or supplement (41). Furthermore, prolonged TRE treatment was not found to have adverse metabolic effects (12). In addition, TRE could be administered orally. Although the human and mouse intestine expresses trehalase, an enzyme that degrades TRE, a small percentage of TRE can pass the intestinal barrier and reach the bloodstream and different organs (12,15).

STUDY LIMITATIONS. We did not apply a correction for multiple testing to our statistical analyses. All of the analyses were performed to test specific hypotheses, and the results had biological explanations. In several cases, multiple analyses were performed to test the same hypothesis in different ways, and the results of these multiple analyses were consistent. For this reason, we believe we could exclude the possibility that our results might be affected by a multiple testing bias.

CONCLUSIONS

Our study demonstrated that oral administration of TRE reduced MI-induced cardiac remodeling and dysfunction through the activation of autophagy (Online Figure 9). Our results suggested that TRE might be a potentially useful pharmacological agent to activate autophagy and reduce cardiac remodeling and heart failure. Additional studies are encouraged to further validate this possibility, including other models of heart diseases. In addition, our results indicated that TRE significantly increases mitophagy. It will be interesting to study in the future whether TRE administration limits the development of mitochondrial dysfunction during stress in the heart.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Oral administration of the nonreducing disaccharide, TRE, reduces adverse post-infarction ventricular dysfunction in mice through activation of autophagy.

TRANSLATIONAL OUTLOOK: Clinical studies are warranted to assess the potential therapeutic usefulness of TRE in patients at risk of developing heart failure following ischemic injury.

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KEY WORDS autophagy, cardiac remodeling, heart failure, trehalose, ventricular function

APPENDIX For supplemental figures, please see the online version of this paper.