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Exercise training restores IGFIR survival signaling in D-galactose induced-aging rats to suppress cardiac apoptosis

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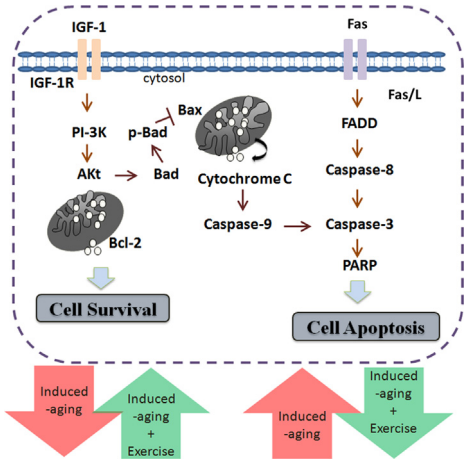
GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: Insulin-like growth factor-I receptor (IGF1R) mediated survival signaling is a crucial mechanism for cellular endurance and a potential indicator of recuperation in deteriorating hearts.

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Objective: This study evaluates the impact of long-term exercise training in enhancing cardiac survival mechanism in D-galactose-induced toxicity associated aging rats.
Methods: Forty-eight male SD-rats were segregated into 4 groups (n=9) and were named as control, exercise training groups, aging group and aging group with exercise training. Aging was induced by intraperitoneal (IP) D-galactose (150 mL/kg) injection for 8 weeks and for exercise training, the rats were left to swim in warm water for 60 min every day and 5 times/week. Western blotting of proteins from the left ventricles was performed to identify the modulations in the survival signaling. Tissue sections were analyzed to determine the extent of fibrosis and apoptosis.
Results: Western-blot analysis performed on the excised left ventricles (LV) showed that proteins of the cardiac survival pathway including IGF1R and Akt and the pro-survival Bcl-2 showed significant decrease in the aging group, whereas the levels were restored in the aging rats subjected to exercise training. In addition, aging groups showed increased interstitial space and collagen accumulation. Further, TUNEL assay showed higher number of apoptotic cells in the LV of aging group, which was correlated with increase in the proteins involved in FAS-FADD-dependent apoptosis. However, these aging associated effects were ameliorated upon exercise training in the D-galactose-induced aging rats that showed elevated IGF1R/Akt signaling.
Conclusion: The results suggest that IGFIR survival signaling cascadeis elevated in following long-term exercise training and thereby provide cardio-protective benefits in D-galactose induced aging rats.

Introduction

Aging is a complicated biological process that causes a gradual but steady decline in the normal physiological and biochemical functions. Successful advancements in the health care has somewhat resulted in an increase in the population of the aging society. Lack of physical activity may also associate with aging with corresponding decline in physical function. Aging causes substantial impact on normal health which include disorders such as muscle atrophy [1], Alzheimer's disease [2], and cardiovascular diseases [3]. Cardiovascular diseases are a major cause of death worldwide, and increase in the global aging population has also led to increase in the mortality due to cardiovascular diseases which is estimated to reach 23.3 million people by 2030 [4].

Exercise training is considered as an efficient strategy in the treatment of cardiovascular diseases [5,6]. Exercise improves the antioxidant capacity and mitochondrial viability in the heart cells and cardio-protection of exercise is known to be correlated with physiological cardiac growth that is distinct from pathological hypertrophy [7,8]. Exercise training in diabetic murine models has shown to provide PGC-1α and Akt mediated cardioprotective effects [7,9]. Mitochondria are responsible to address various physiological or metabolic demands, and they play an important role in cellular proliferation [10]. Various studies have confirmed that the aging disorders are associated with the loss of mitochondrial homeostasis and deterioration of tissue mitochondrial function [11,12]. Imbalance in mitochondrial function leads to increase in cellular apoptotic events [13]. When the mitochondrial membrane potential is lost, the mitochondrial cytochrome *c* is released to cytosol which subsequently results in cellular apoptosis [14]. Further, circulating insulin-like growth factor I (IGF-1) levels are negatively correlated with cardiovascular risks and is considered as a prognostic indicator in conditions such as ischemic heart disease but most importantly, reduced levels of IGF-1 in aged persons increases the risk for heart failure [15–17]. IGF-1 signaling is transduced by its transmembrane tyrosine kinase receptor IGF1R. Docking of IGF-1 to its receptor results in the activation of its downstream signaling in cardiomyocytes by triggering receptor autophosphorylation [18,19]. The downstream signaling cascades subsequently regulate metabolism, cellular proliferation, differentiation, cellular hypertrophy and cell survival in heart [15]. IGF1R mediated activation of Akt survival pathway and the ERK pathway are well studied in cardiomyocytes for their involvement in cell survival [20,21]. Activated Akt may exhibit direct inhibition effects on pro-apoptotic Bcl-2 family proteins [22,23]. In addition, recent studies show that MitoKATP-mediated mitochondrial translocation of pAkt potentially provide cardio-protection against hypoxia-induced apoptosis [24]. For its importance for being a survival factor, it is imminent to determine if IGF1R activation can be restored in aging condition following exercise.

In the present study, the effects of exercise on D-galactose induced aging associated reduction in IGF1R function and cardiac damages were determined on Sprague-Dawley rats. The results show a positive influence of IGF1R in aging animals.

Methods and Material

Animal experiments

Three weeks old male Sprague-Dawley (SD) rats were procured from BioLASCO (A Charles River Licensee Corporation, Yi-Lan, Taiwan). The rats underwent adaptation for a week. The rats were provided with standard laboratory diet (Lab Diet 5001; PMI Nutrition International Inc., Brentwood, MO, USA) and drinking water was provided *ad libitum* and were properly housed in optimized temperature (24 ± 2 °C and humidity 55 ± 10%). Rats were segregated into different groups (n = 9): Control group, normal rats with swimming exercise, aging, aging group with swimming exercise. In order to induce aging, the rats were injected with D-galactose IP (150 mL/kg of body weight) for 8 weeks. The control and the exercise-training group were administered with 0.9% physiological saline. The swimming training was performed following previous report by Hart, et al [25]. In the first two weeks, the normal rats from exercise training group and aged rats from the aging group with swimming exercise were left for swimming for 20 min/day, 5 times/week. The duration of swimming was extended to 30 min/-day starting from the 3rd week and to 60/min during fourth to eighth wee. The rats were left for swimming in 50 cm deep of water maintained at 35 ± 1 °C [26] individually in a 60 × 90 cm water tub. All protocols were approved by the Institutional Animal Care and Use Committee of China Medical University, Taichung, Taiwan. The study followed the principles of laboratory animal care [27].

Western blotting analysis

Protein extracts were derived upon homogenization using tissues in a lysis buffer (100 mg tissue/mL buffer) containing 0.05 M Tris-HCl (pH 7.4), 0.15 M NaCl, deoxycholic acid (0.25%), NP-40 (1%), 1 mM EDTA. After centrifuging the homogenates at

12,000*g* for 40 min the supernatants were collected and stored at −80 °C. The protein concentration was determined by Lowry method and the Western blotting analysis was performed following methods mentioned in other reports with slight modification [28]. The PVDF membranes with transferred proteins were blocked in 3% bovine serum albumin (BSA) in TBS buffer. The primary antibodies Fas-L(SC-956), FADD(SC-6035), Caspase-8 (SC-6134), Bax (SC-526), α-tubulin(SC-5286), β-actin (SC-47778), p-IGF1R (sc-101703), Akt (SC-5298) and Bcl-2 (SC-7382) were purchased from Santa Cruz Biotechnology (California, USA); IGF1R (ab19675) was from Abcam Biotechnology (Cambridge, UK) and p-Akt (#9275), Cleaved caspase-3 (#9664) and PARP (#9542) were from Cell Signaling technologies (Maryland, USA)., Cell Signaling, Maryland, USA). Appropriate secondary antibodies were used and the immunoblots were visualized and documented with Fujifilm LAS-4000 (GE healthcare UK limited, Buckinghamshire, UK).

Masson's trichrome staining and TUNEL assay

Terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) assay for the tissue sections was performed as mentioned in the previous report [29]. Briefly, the de-waxed and rehydrated tissue sections were treated with proteinase K followed by permeabilization solution and then with TUNEL reagent (Roche Applied Science, Indianapolis, IN). The nucleus was then stained using 4, 6-diamidino-2-phenylindole (DAPI). The TUNEL-positive nuclei were illuminated in green and DAPI stained nucleus were in blue. Photomicrographs were obtained using fluorescent microscope (DP 74, Olympus, Tokyo, Japan). Masson's trichrome was performed following procedure mentioned in previous report [30] and the de-waxed and rehydrated tissue sections were placed in freshly prepared Weigert's Hematoxylin reagent for 10 min and then in Briebrch Scarlet for 10 min followed by placing in phosphotungstic phophomolybdic acid for 10 min. After applying anilin blue solution for 10 min and in 1% glacial acetic acid, the slides were dehydrated with graded ethanol and photomicrographs were captured in Zeiss Axiophot microscope (Zeiss, Oberkochen, Germany).

Statistical analysis

The results are shown as means ± SEM obtained from 3 independent experiments. The differences among the groups were analyzed by one-way ANOVA analysis using Graphpad prism software (GraphPad Software Inc, San Diego, CA, USA) followed by Tukey's post hoc test and all results were quantified using ImageJ software (NIH, MD). $p < 0.05$ was considered statistically significant.

Results

Exercise training attenuates aging-associated cardiac apoptosis

TUNEL assay was performed to determine if D-galactose induced-aging could induce cardiac apoptosis and if exercise training could help overcome the aging-associated cardiac apoptosis. TUNEL assay on the left ventricular section from aging-induced rats show that increased number of apoptotic nuclei compared to the control group. The number of TUNEL-positive cardiac cells was 6.5 folds higher in D-galactose induced-aging rats and exercise treatment in the aging rats reduced the apoptosis by 66% with respect to the number of apoptotic cells (Fig. 1).

Effects of aging and exercise training on cardiac fibrosis

Masson's Trichrome staining of the heart sections show that D-galactose induced-aging rats displayed large amounts of collagen accumulation which was reduced in aging rats with swimming

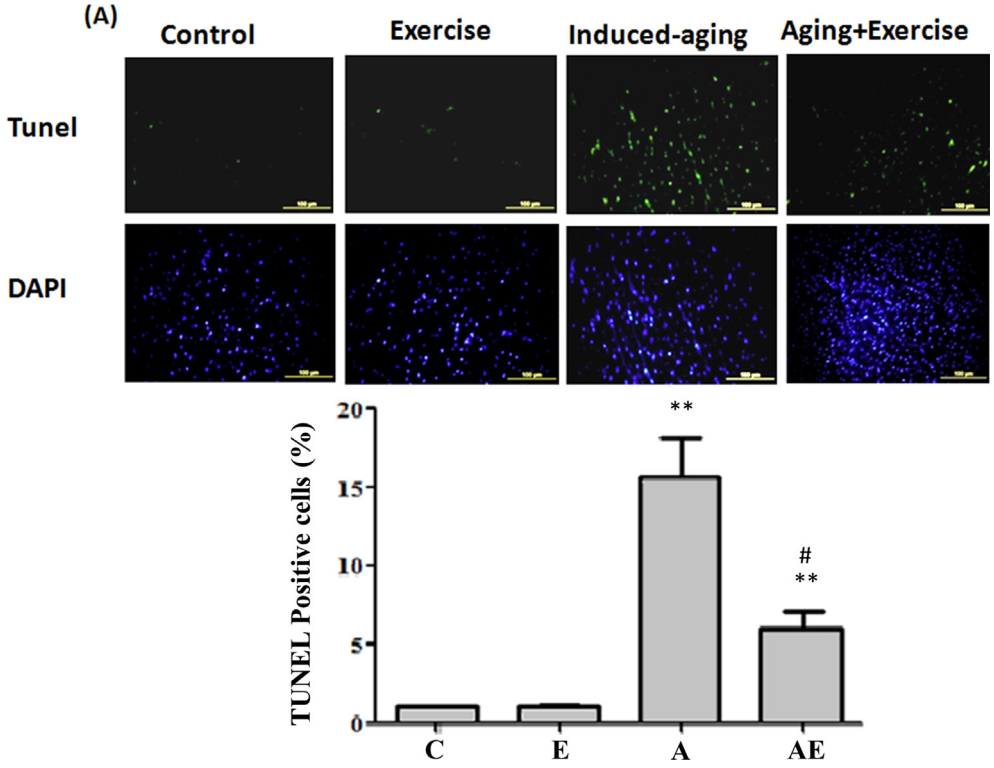

Fig. 1. Exercise attenuates aging-associated cardiac apoptosis. (A) Representative microscopic images show stained apoptotic cells in heart tissue sections of rats from different groups (Control and Exercise: rats under exercise training, induced-aging: D-galactose induced aging rats and Aging + Exercise: D-galactose induced aging rats under exercise training). Percentage of DAPI stained (upper panels, blue spots) nuclei and TUNEL stained (lower panels, green spots, x400) nuclei are presented in bars (n≧3 in each group). $^{**}P < 0.01$ denotes significant differences compared to that of the Control group. $\#P < 0.05$ denotes significant differences compared to that of the Induced-aging group.

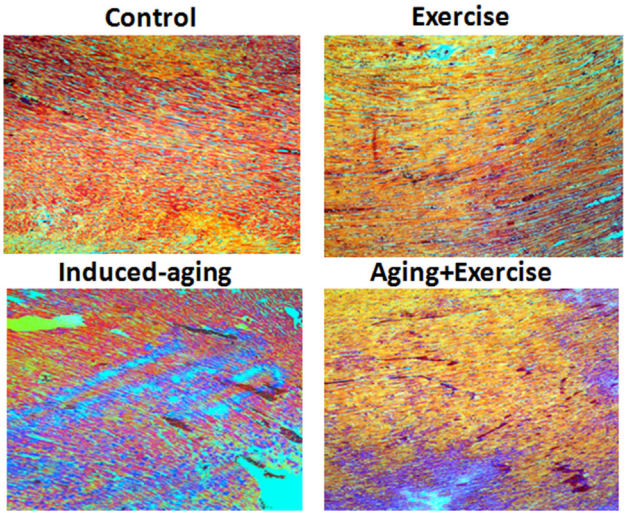

Fig. 2. Exercise training attenuates aging associated cardiac fibrosis. Representative Masson's trichrome stain showing Collagen accumulation (blue color) in heart tissue section of Control, exercise training, aging and aging with exercise group rats.

(Fig. 2). The results therefore show that exercise training in aging group potentially restore the cardiac contractile function due to reduction in the cardiac remodeling.

Exercise training on D-galactose-induced modulations in cardiac extrinsic apoptotic pathway

To determine the changes in the cardiac Fas receptor-involved apoptosis mechanism in the aging model after exercise training, Fas ligand (Fas-L), FADD and active caspase-8 levels were checked by Western blot analysis. The results reveal that Fas-L and FADD increased significantly in the aging rats (Fig. 3) and thereby revealed the involvement of these factors in the aging-associated apoptosis. Further, while exercise training did not cause any notable changes in the apoptosis events in normal rats, exercise training in the aging group were significantly reduced up on exercise training as evident the active caspase-8 protein levels. Therefore, 8 weeks of exercise training potentially ameliorates aging associated extrinsic apoptosis.

Modulations in aging associated cardiac intrinsic apoptotic pathway

To determine the changes in the mediators of cardiac mitochondria-dependent apoptotic pathways, the levels of Bax, cleaved caspase-3 and PARP were analyzed by Western blotting (Fig. 4). The results show that cardiac Bax, cleaved caspase-3 and PARP levels were significantly elevated in the aging group. Meanwhile, there was no notable change in Bax, cleaved caspase-3 and PARP levels between the control group and the normal rats with exercise training. But, aging rats with exercise training showed a strong reduction in the level of cleaved caspase-3. The results therefore reveal that exercise training effectively suppress aging associated intrinsic apoptosis.

Effect of exercise training on cardiac pro-survival pathway

Further to ascertain the changes in cardiac IGF1R/AKT survival cascade in the aging rats caused due to exercise training, the pro-survival associated proteins of the heart were measured by western blotting (Fig. 5). The cardiac p-IGF1R, p-Akt and Bcl-2 protein levels were significantly downregulated in the aging group. However, exercise training showed significant enhancement in the levels of survival proteins like IGF1R, p-Akt & Bcl-2 in the aging rats. The results therefore highlight that IGF1R associated survival cascade is an important prognostic even in exercise induced protection against the pathological phenomenon of aging.

Discussion

Various alternative approaches have been demonstrated to deliver cardioprotective effects against numerous pathological conditions [30–37]. According to American College of Sports Medicine and the American Heart Association exercise training provide car-

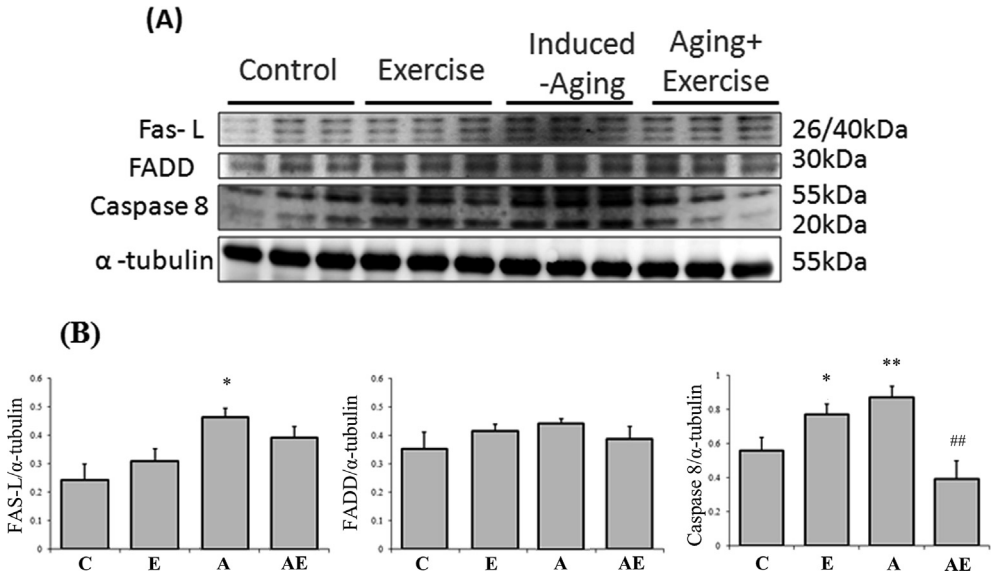

Fig. 3. Effect of exercise training on proteins involved in extrinsic apoptosis. (A) Representative Western blots show the changes in the protein levels of Fas-L, FADD and Caspase-8 in the left ventricle tissue in from different groups (Control and Exercise: rats under exercise training, induced-aging: D-galactose induced aging rats and Aging + Exercise: D-galactose induced aging rats under exercise training). (C) Bars represent the ratio of band intensities with respect to that of the internal control. The data represents mean values ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ represent significant differences with respect to control group. $\#\#P < 0.01$ denotes significant differences with respect to aging group.

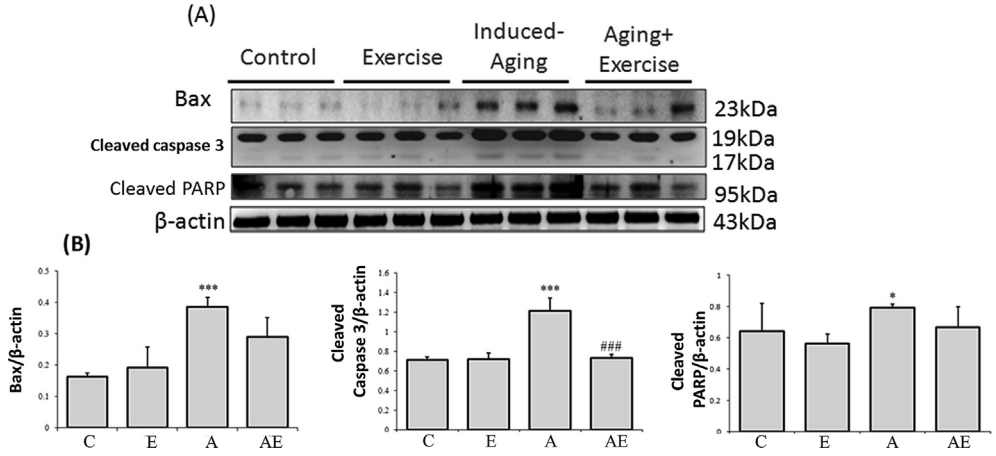

Fig. 4. Effect of exercise training on proteins of intrinsic apoptosis. (A) Representative protein products of Bax, Cleaved Caspase-3 and PARP from left ventricles of Control (C), exercise training (E), aging (A) and aging with exercise (AE) group rats were measured by Western blotting analysis. The β-actin was used as an internal control. (C) Bars represent the relative fold changes in protein levels representing mean values ± SEM. *P < 0.05, and ***P < 0.001represent significant differences with respect to control group. ###*P* < 0.001 represents significant differences with respect to aging group.

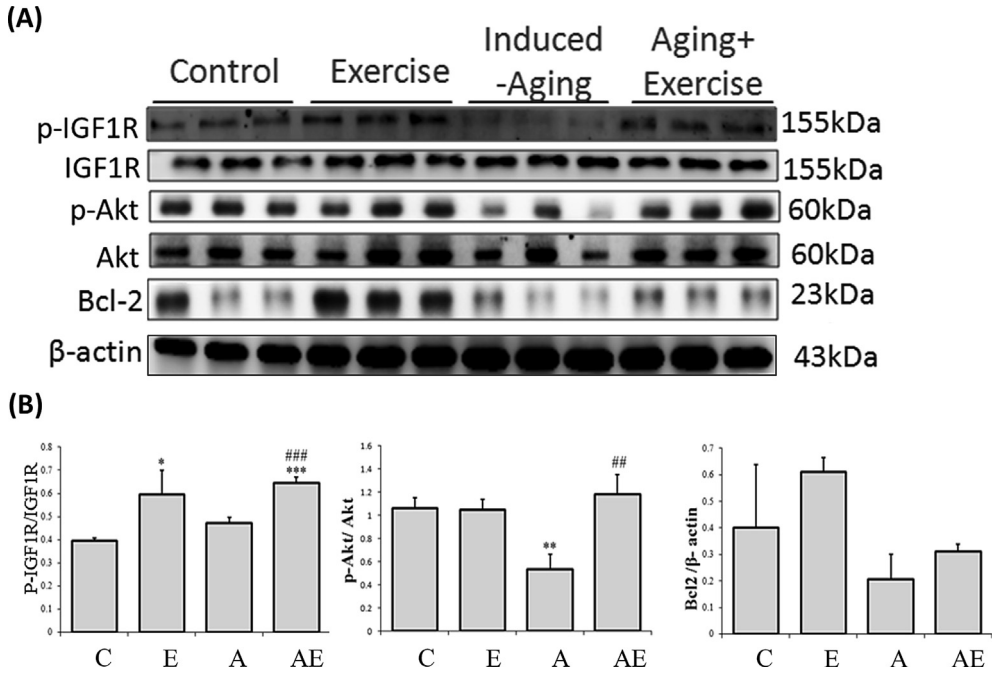

Fig. 5. Exercise reboots survival signaling in the cardiac cells of aging rats (A) representative protein products of IGF1R, p-IGF1R, Akt, p-Akt and Bcl-2 extracted from the left ventricles of Control (C), exercise training (E), aging (A) and aging with exercise (AE) group rat hearts were measured by Western blotting analysis. The β-actin was used as an internal control. (C) Bars represent the relative fold changes in protein levels representing mean values ± SEM. *P < 0.05, **P < 0.01 and ***P < 0.001significant differences with respect to control group. ##P < 0.01 and ###P < 0.001 significant differences with respect to aging group.

diacprotection in conditions like hypertension, coronary artery disease and it is also widely known to attenuate ischemia-reperfusion injury in aged adults [38,39].

In this study, nuclear staining in hearts of D-galactose-induced aging mice showed that there was a 15% increase in the number of apoptotic nuclei in cardiac cells. However, the D-galactose-induced aging mice with exercise training, the cells apoptosis found to be reduced to 3%. Various study show that apoptotic rates of 2–12% affect the physiological function of the heart and causeir-reversible damage to the heart and affects systemic blood supply [40].

Our previous study also demonstrated that aging triggers cardiac cell death mediated by Fas-L that binds to its receptor Fas. Fas-L with Fas will activate its death-domain and downstream protein FADD and upon the release of pro-caspase-8 they combine with FADD in the cytoplasm to activate caspase-8 that directs re-activation of caspase-3 protein and induce the apoptosis program. Activation of Caspase-8 could also lead Bid into t-Bid. The t-Bid gets embedded into the mitochondrial outer membrane, causing mitochondrial released of cytochrome-*c* which induces Caspase-3 activation by Caspase-9 and triggers cell apoptosis. In our results, the Caspase-8 and Fas-FADD were augmented in D-galactose induced aging group and were reduced in exercise training group [41]. The results suggest that D-galactose induced aging rats, similar to natural aging rats, display cardiac physiological changes and increased cardiac cell death (Fig. 6). Suppression of Fas-FADD path-

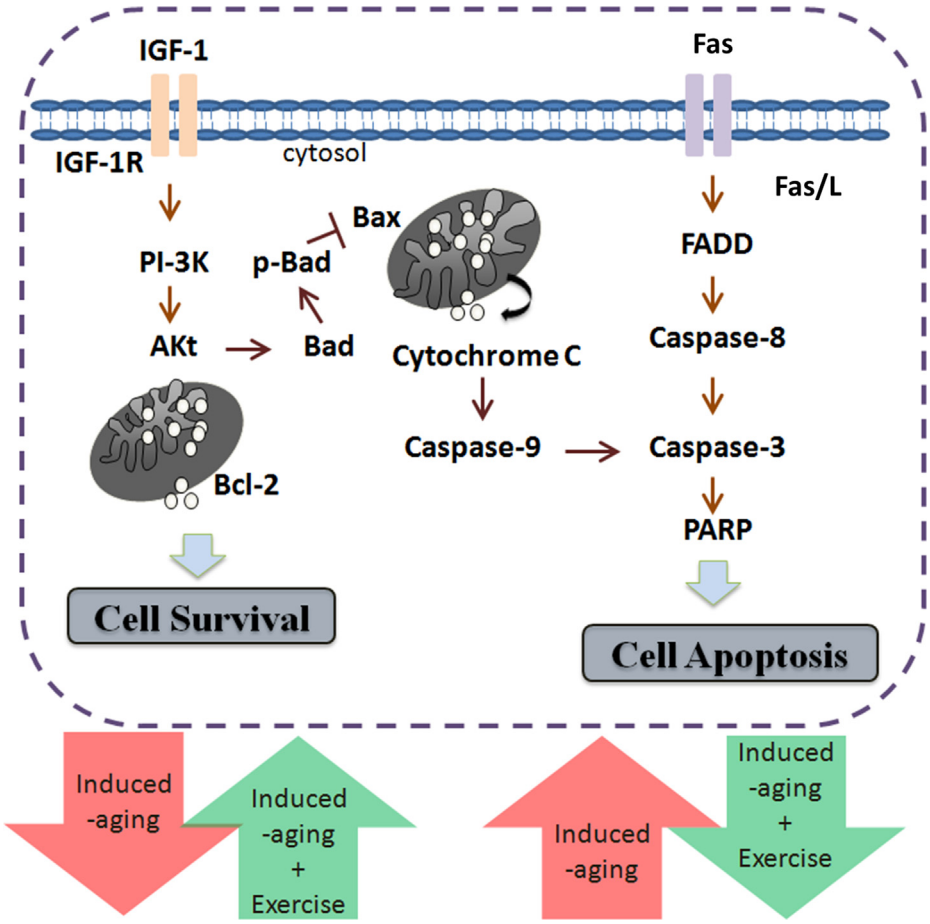

Fig. 6. Schematic representation on the molecular events involved in cardio-protection provided by exercise training.

way by exercise training is a potential way for controlling myocardial cellular apoptosis during the progression of cardiac disease. In line with other reports, our results show that D-galactose induced aging causes a notable increase in cleaved Caspase-3 levels and PARP cleavage [42,43]. It should be also noted that, Caspase-3 and PARP remain inactive in control rats and in normal rats with exercise.

The PI3K-Akt signal pathway is a notable survival mechanism that could be regulated by IGF1R. Enhancement of IGFIR and its associated survival factors is considered as a hallmark of efficient cardioprotection against various pathological models of cardiac defects [30–32,35,37,44]. IGF-I and its associated pro-survival proteins p-PI3k, p-Akt, Bcl-2 and Bcl-xL were seen to be elevated in STZ induced diabetic models as well, indicating their compensatory survival mechanism to suppress apoptosis under various stresses [15,29,45]. In our study, p-Akt/Akt were significantly elevated in young mice with exercise training and given exercise training after D-galactose-induced aging mice. These results suggested that regardless of young or old mice, PI3K-Akt signal cascade plays a central role in cellular survival mechanism and exercise training is an effective means in maintaining the PI3K-Akt activation *in vivo*. Further, IGF1R survival mechanism could be a possible hallmark for the beneficial effects in heart conferred upon exercise training.

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Author contributions

Ing-Shiow Lay and Chih-Yang Huang designed the study. Chih-Yang Huang, Tsung-Jung Ho and Cecilia Hsuan Day verified the data. Marthandam Asokan Shibu drafted the manuscript. Wei-Wen Kuo performed statistical analysis. Shiu-Min Cheng, Bo Ban, Shulin Wang, Qiaowen Li proof read the manuscript. Chih-Yang Huang obtained funding and provided resources for the study.

Compliance with Ethics Requirements

Animal experiments conform to internationally accepted standards and have been approved by the appropriate institutional review body.

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.

Acknowledgment

The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation, and the results of the present study do not constitute endorsement by ACSM

References

[1] Ariznavarreta C, Castillo C, Segovia G, Mora F, Azcoitia I, Tresguerres JA. Growth hormone and aging. Homo: internationale Zeitschrift fur die vergleichende Forschung am Menschen 2003;54:132–41.
[2] Pratico D. Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows. Ann N Y Acad Sci 2008;1147:70–8.
[3] Lubos E, Handy DE, Loscalzo J. Role of oxidative stress and nitric oxide in atherothrombosis. Front Biosci 2008;13:5323–44.
[4] Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006;3:e442.
[5] Pagan LU, Damatto RL, Cezar MDM, Lima ARR, Bonomo C, Campos DHS, et al. Long-term low intensity physical exercise attenuates heart failure development in aging spontaneously hypertensive rats. Cell Physiol Biochem 2015;36:61–74.
[6] Wilson GA, Wilkins GT, Cotter JD, Lamberts RR, Lal S, Baldi JC. HIIT Improves left ventricular exercise response in adults with Type 2 diabetes. Med Sci Sports Exerc 2019;51:1099–105.
[7] Wang H, Bei Y, Lu Y, Sun W, Liu Q, Wang Y, et al. Exercise prevents cardiac injury and improves mitochondrial biogenesis in advanced diabetic cardiomyopathy with PGC-1α and Akt activation. Cell Physiol Biochem 2015;35:2159–68.
[8] Hinkley JM, Morton AB, Ichinoseki-Sekine N, Huertas AM, Smuder AJ. Exercise training prevents doxorubicin-induced mitochondrial dysfunction of the liver. Med Sci Sports Exerc 2019;51:1106–15.
[9] Tao L, Bei Y, Lin S, Zhang H, Zhou Y, Jiang J, et al. Exercise training protects against acute myocardial infarction via improving myocardial energy metabolism and mitochondrial biogenesis. Cell Physiol Biochem 2015;37:162–75.
[10] Moyes CD, Mathieu-Costello OA, Tsuchiya N, Filburn C, Hansford RG. Mitochondrial biogenesis during cellular differentiation. Am J Physiol 1997;272:C1345–51.
[11] Emerit J, Edeas M, Bricaire F. Neurodegenerative diseases and oxidative stress. Biomed Pharmacother = Biomed Pharm 2004;58:39–46.
[12] Kwong LK, Sohal RS. Age-related changes in activities of mitochondrial electron transport complexes in various tissues of the mouse. Arch Biochem Biophys 2000;373:16–22.
[13] Phaneuf S, Leeuwenburgh C. Apoptosis and exercise. Med Sci Sports Exerc 2001;33:393–6.
[14] Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell 1996;86:147–57.
[15] Troncoso R, Ibarra C, Vicencio JM, Jaimovich E, Lavandero S. New insights into IGF-1 signaling in the heart. Trends Endocrinol Metab: TEM 2014;25:128–37.
[16] Hsieh CH, Pai P, Wu JP, Ho TJ, Wu CH, Shibu MA, et al. Activation of IGF-I survival signaling and its compensative inhibition of the cardiac apoptosis on carotid arteries balloon-injured rat hearts. Chinese J Physiol 2017;60:166–73.
[17] Vasan RS, Sullivan LM, D'Agostino RB, et al. Serum insulin-like growth factor i and risk for heart failure in elderly individuals without a previous myocardial infarction: the framingham heart study. Ann Intern Med 2003;139:642–8.
[18] Adams TE, Epa VC, Garrett TPJ, Ward CW. Structure and function of the type 1 insulin-like growth factor receptor. Cell Mol Life Sci CMLS 2000;57:1050–93.
[19] Ro Foncea, Andersson M, Ketterman A, Blakesley V, Sapag-Hagar M, Sugden PH, et al. Insulin-like growth factor-I rapidly activates multiple signal transduction pathways in cultured rat cardiac myocytes. J Biol Chem 1997;272:19115–24.
[20] Kim J, Wende AR, Sena S, Theobald HA, Soto J, Sloan C, et al. Insulin-like growth factor I receptor signaling is required for exercise-induced cardiac hypertrophy. Mol Endocrinol 2008;22:2531–43.
[21] Foncea R, Gálvez A, Pérez V, Morales MP, Calixto A, Meléndez J, et al. Extracellular regulated kinase, but not protein kinase C, is an antiapoptotic signal of insulin-like growth factor-1 on cultured cardiac myocytes. Biochem Biophys Res Commun 2000;273:736–44.
[22] Lin KH, Kuo WW, Jiang AZ, Pai P, Lin JY, Chen WK, et al. Tetramethylpyrazine ameliorated hypoxia-induced myocardial cell apoptosis via HIF-1α/JNK/p38 and IGFBP3/BNIP3 inhibition to upregulate PI3K/Akt survival signaling. Cell Physiol Biochem 2015;36:334–44.
[23] Liu S-P, Shibu MA, Tsai F-J, Hsu Y-M, Tsai C-H, Chung J-G, et al. Tetramethylpyrazine reverses high-glucose induced hypoxic effects by negatively regulating HIF-1α induced BNIP3 expression to ameliorate H9c2 cardiomyoblast apoptosis. Nutr Metab (Lond) 2020;17:12-.
[24] Song HP, Chu ZG, Zhang DX, Dang YM, Zhang Q. PI3K–AKT pathway protects cardiomyocytes against hypoxia-induced apoptosis by MitoKATP-mediated mitochondrial translocation of pAKT. Cell Physiol Biochem 2018;49:717–27.
[25] Hart KJ, Shaw JM, Vajda E, Hegsted M, Miller SC. Swim-trained rats have greater bone mass, density, strength, and dynamics. J Appl Physiol (Bethesda, Md.: 1985) 2001;91:1663–8.
[26] Arai I, Tsuyuki Y, Shiomoto H, Satoh M, Otomo S. Decreased body temperature dependent appearance of behavioral despair in the forced swimming test in mice. Pharmacol Res 2000;42:171–6.
[27] Guide for the Care and Use of Laboratory Animals, Washington DC: © National Academy of Sciences; 2011.
[28] Wang ZH. Anti-glycative effects of asiatic acid in human keratinocyte cells. BioMedicine 2014;4:19.
[29] Huang PC, Wang GJ, Fan MJ, Asokan Shibu M, Liu YT, Padma Viswanadha V, et al. Cellular apoptosis and cardiac dysfunction in STZ-induced diabetic rats attenuated by anthocyanins via activation of IGFI-R/PI3K/Akt survival signaling. Environ Toxicol 2017;32:2471–80.
[30] Hsieh YL, Shibu MA, Lii CK, Viswanadha VP, Lin YL, Lai CH, et al. Andrographis paniculata extract attenuates pathological cardiac hypertrophy and apoptosis in high-fat diet fed mice. J Ethnopharmacol 2016;192:170–7.
[31] Lee SNC, Ho TJ, Shibu MA, Day CH, Viswanadha VP, Lai CH, et al. Protective effects of electroacupuncture at LR3 on cardiac hypertrophy and apoptosis in hypertensive rats. Acupunct Med 2016;34:201–8.
[32] Liao HE, Shibu MA, Kuo WW, Pai PY, Ho TJ, Kuo CH, et al. Deep sea minerals prolong life span of streptozotocin-induced diabetic rats by compensatory augmentation of the IGF-I-survival signaling and inhibition of apoptosis. Environ Toxicol 2016;31:769–81.
[33] Chen YF, Shibu MA, Fan MJ, Chen MC, Viswanadha VP, Lin YL, et al. Purple rice anthocyanin extract protects cardiac function in STZ-induced diabetes rat hearts by inhibiting cardiac hypertrophy and fibrosis. J Nutr Biochem 2016;31:98–105.
[34] Asokan Shibu M, Kuo WW, Kuo CH, Day CH, Shen CY, Chung LC, et al. Potential phytoestrogen alternatives exert cardio-protective mechanisms via estrogen receptors. BioMedicine 2017;7:11.
[35] Shibu MA, Kuo CH, Chen BC, Ju DT, Chen RJ, Lai CH, et al. Oolong tea prevents cardiomyocyte loss against hypoxia by attenuating p-JNK mediated hypertrophy and enhancing P-IGF1R, p-akt, and p-Bad(ser136) activity and by fortifying NRF2 antioxidation system. Environ Toxicol 2018;33:220–33.
[36] Shibu MA, Agrawal DC, Huang C-Y. Mushrooms: a pandora box of cardioprotective phytochemicals. In: Agrawal DC, Tsay H-S, Shyur L-F, Wu Y-C, Wang S-Y, editors. Medicinal plants and fungi: recent advances in research and development. Singapore: Springer Singapore; 2017. p. 337–62.
[37] Marthandam Asokan S, Mariappan R, Muthusamy S, Velmurugan BK. Pharmacological benefits of neferine - a comprehensive review. Life Sci 2018;199:60–70.
[38] Lee Y, Min K, Talbert EE, Kavazis AN, Smuder AJ, Willis WT, et al. Exercise protects cardiac mitochondria against ischemia-reperfusion injury. Med Sci Sport Exer 2012;44:397–405.
[39] Nelson ME, Rejeski WJ, Blair SN, Duncan PW, Judge JO, King AC, et al. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 2007;39:1435–45.
[40] van Empel VPM, Bertrand ATA, Hofstra L, Crijns HJ, Doevendans PA, De Windt LJ. Myocyte apoptosis in heart failure. Cardiovasc Res 2005;67:21–9.
[41] Huang C-Y, Yang A-L, Lin Y-M, Wu F-N, Lin JA, Chan Y-S, et al. Anti-apoptotic and pro-survival effects of exercise training on hypertensive hearts. J Appl Physiol 2012;112:883–91.
[42] Ali T, Badshah H, Kim TH, Kim MO. Melatonin attenuates D-galactose-induced memory impairment, neuroinflammation and neurodegeneration via RAGE/NF-K B/JNK signaling pathway in aging mouse model. J Pineal Res 2015;58:71–85.
[43] Bo-Htay C, Palee S, Apaijai N, Chattipakorn SC, Chattipakorn N. Effects of d-galactose-induced ageing on the heart and its potential interventions. J Cell Mol Med 2018;22:1392–410.
[44] Chen PY, Hou CW, Shibu MA, Day CH, Pai P, Liu ZR, et al. Protective effect of Co-enzyme Q10 On doxorubicin-induced cardiomyopathy of rat hearts. Environ Toxicol 2016.
[45] Huynh K, McMullen JR, Julius TL, Tan JW, Love JE, Cemerlang N, et al. Cardiac-specific IGF-1 receptor transgenic expression protects against cardiac fibrosis and diastolic dysfunction in a mouse model of diabetic cardiomyopathy. Diabetes 2010;59:1512–20.