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Cardioprotective effect of a moderate and prolonged exercise training involves sirtuin pathway

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ABSTRACT

Aim: To investigate the cardioprotective effects of prolonged and moderate exercise training on cellular and molecular events early after myocardial infarction.

Materials and methods: Male Wistar rats were divided in sedentary or exercised group; both groups underwent to a myocardial infarction. All the molecular and immunohistochemical analyses on hearts of sedentary and exercised rats were performed 48 h after surgical procedure. SIRT1 and SIRT3 expression were measured and two of the pathways activated by sirtuins, p53-induced apoptosis and Forkhead boxO (FOXO)3a-induced oxidative stress, were investigated. All the experiments were performed also in presence of the SIRT inhibitor, EX527.

Key findings: Fourty-eight hours post myocardial infarction, exercise training induced the activation of SIRT1 and SIRT3 pathway reducing cardiomyocytes apoptosis and oxidative damage. Molecular data were confirmed by immunohistochemical evaluations. These effects are more evident in border infarcted zone than in the remote myocardium.

Significance: Exercise training is a non-pharmacological prevention strategy in cardiovascular diseases and the sirtuins family seems to be as novel and attractive target in cardioprotection.

1. Introduction

Coronary artery disease and acute myocardial infarction (MI) are leading cause of morbidity and mortality worldwide [1]. Although the cellular events that mediate cardiac damage after MI are complex and not fully understood, an excessive generation of free radicals and apoptosis are the main determinants of cardiomyocyte loss [2,3].

In recent years, clinical and experimental studies have highlighted the role of exercise training as an adjuvant therapy for a variety of cardiovascular diseases, despite to date there are no randomized clinical trials testing this effect on cardiac events such as MI [4,5].

Moreover, although the cardioprotective effect of exercise training in cardiovascular diseases is well documented, the underlying molecular pathways need further investigations. Certainly, exercise increases the expression of numerous cardiac genes and there is an evidence that exercise-induced activation of anti-oxidant system contributes to its cardioprotective effect [6].

Among these mechanisms, the involvement of sirtuins in the beneficial effects of exercise training seems to be crucial [7] and we have previously demonstrated that a moderate exercise training increased sirtuin (SIRT) 1 activity [8].

Sirtuins are a highly conserved family of (NAD)+ -dependent histone deacetylase which activity is linked to a variety of pathophysiological processes, such as caloric restriction, apoptosis, and oxidative stress [9,10].

Several experimental models of cardiovascular diseases highlighted the beneficial effects of sirtuins in cardiomyocytes survival and in tolerance to oxidative stress [11,12].

In particular, the role of SIRT1 in cardioprotection has been demonstrated in SIRT1 knockout mice by showing an exacerbated ischemia/reperfusion-induced myocardial injury [13].

SIRT1 catalyzes the deacetylation of different proteins including the tumor suppressor p53 which inhibits the downstream proapoptotic gene expression and then the activation of the apoptotic cascade [14].

Another target directly deacetylated by the sirtuins is the Forkhead boxO (FoxO) [15]; in particular, SIRT3 increases the FoxO3a-induced antioxidant genes as manganese superoxide dismutase (MnSOD) and catalase [16,17]. Several experimental studies have demonstrated that

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SIRT3 is abundantly expressed in the heart and that it plays a cardioprotective role; mice lacking SIRT3 showed an enhanced susceptibility to cardiac diseases [18,19].

The relationship between volume and intensity of exercise and benefits obtained has been investigated in clinical settings, and the evidence indicates that low and moderate exercise training can be beneficial for the majority of cardiovascular patients [4].

However, the effects of a prolonged moderate exercise on the sirtuin pathway and the early molecular and cellular events after an acute MI are not known.

Therefore, the aim of the present study was to investigate the involvement of the SIRT1 and SIRT3 pathway in the exercise training-induced cardioprotection in infarcted rats.

To this end we analyzed the anti-apoptotic and anti-oxidant effects of sirtuins and, to further investigate the possibility of their involvement, we used the sirtuins inhibitor EX527.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (225–250 g) were purchased from Envigo (San Piero al Natisone, Italy) and were housed individually under controlled illumination (12:12 h light/dark cycle; light on 06.00 h) and environmental conditions (room temperature 20–22 °C, humidity 55–60%) for at least 1 week before the beginning of experiments. Chow and tap water were available *ad libitum*. Animal care was in compliance with the IASP and European Community (E.C. L358/1 18/12/86) guidelines. All researches involving animals were performed in accordance with the European directive 86/609/EEC governing animal use and protection and in compliance with Italian guidelines (D.L. 116/92). All the experimental procedures were also approved by the Animal Ethics Committee of the University of Campania, "Luigi Vanvitelli", Naples.

2.2. Training protocol

Fourty-seven rats (n = 47) were randomly allocated into three main groups: sedentary rats (SED, n = 20), exercised rats (EX, n = 20) and exercised rats treated with a SIRT1 selective inhibitor, EX527 (EX + INH, n = 7). The training protocol has been described previously [20]. Briefly, EX and EX + INH rats were acclimated to training by walking at a speed of 10 min/day on a treadmill for 2 weeks (Panlab/Harvard Apparatus Treadmills, Holliston, MA, USA). From week 3, training consisted of running of 30 m/min, 45 min/day, 5 days/week, for 6 weeks. A horizontal shock grid that delivered < 1 mA was placed 10 cm from the rear of the chamber to provide stimulus for the animals to run. Sedentary rats spent the same time (*i.e.* 8 weeks) in the cages.

The SIRT1 selective inhibitor, EX527 (1.15 $mgKg^{-1}die^{-1}$), was dissolved in 0.5% DMSO and was administered daily by gavage 30 min before starting the training until the end of the exercise protocol (Fig. 1).

2.3. Surgical procedure

Twenty-four hours after the last session of exercise training rats underwent to myocardial infarction (MI) by surgical occlusion of the left anterior descended (LAD) coronary artery, according to previously described procedures [21]. All rats were sacrificed two days after MI.

2.4. Western blot analysis

The hearts obtained from all rats were further divided in two portions: border (perinfartual) and remote zone.

Total protein extraction and protein content were obtained as described elsewhere [22,23]. Tissues were homogenized on ice in 1 mL RIPA lysis buffer (Tris HCl pH 7.8, 0.1% sodium deoxycholate, 1% SDS, NP-40 1 ×) containing protease and phosphatase inhibitors and the tubes were vigorously shaken at 4 °C for 30 min on a shaking platform. Lysates were centrifuged at 12000 × *g* for 10 min to remove the insoluble debris. After centrifugation the supernatants were frozen in aliquots at −80 °C until use. Protein contents were determined by the Bradford method. Protein samples (40 μg per lane) were separated on denaturing 10% SDS polyacrylamide gel and transferred to a PVDF membrane. Non-specific binding to the membrane was blocked for 1 h at room temperature with 5% milk in T-TBS (Tris buffer saline with 0.1% Tween 20). Membranes were incubated at 4 °C overnight with primary antibody against SIRT1, SIRT3, FOXO-3a (Abcam), Bax, and caspase-3 (Santa Cruz), p53, acetyl-p53 (Lys379) (Cell Signalling), catalase (Sigma). All the antibodies were used 1:1000 5% milk in T-TBS.

The membranes were washed three times with 0.1% T-TBS solution, and then incubated for 1 h at room temperature with a secondary antibody anti-rabbit IgG peroxidase conjugated for SIRT1, SIRT3, FOXO-3a, p53, acetyl-p53(1:10000 in 5% milk in T-TBS, Santa Cruz Biotechnology) and secondary antibody anti-mouse IgG peroxidase conjugated for Bax, Caspase3 and catalase (1:10000 in 5% milk in T-TBS, Santa Cruz Biotechnology). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:1000 in 5% milk in T-TBS, Santa Cruz) polyclonal antibody was used as an internal standard. The immunoreactive bands were visualized using an enhanced chemilumunescence system (SuperSignal West Femto Maximum Sensitivity Substrate, Pierce, Rockford, USA). The protein bands were scanned and quantitated with Gel Doc-2000 (Bio-Rad, Milan, Italy).

2.5. Immunohistochemistry

For fluorescence imaging, formalin-fixed-paraffin-embedded tissue sections were deparaffinized in xylenes and rehydrated in the decreasing concentrations of ethanol. Fragmented DNA was detected with TUNEL (terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick-end-labeling) method using ApoAlert DNA Fragmentation Assay Kit according to manufacturer's instructions (Clontech Laboratories). For the detection of SIRT1, tissue sections were subjected to antigen unmasking protocol using microwave heating in citrate buffer at pH = 6.0 for 10 min. After blocking with 5% donkey serum for 30 min at room temperature, the samples were incubated overnight at 4 °C with an anti-SIRT1 primary antibody (Abcam) at 1:100 dilution followed with a 1 h incubation at 37 °C with a TRITC-conjugated secondary antibody diluted 1:100 (JacksonImmuno). Subsequently, samples were incubated for 1 h at 37 °C with an anti-α-sarcomeric actin (Sigma) antibody diluted 1:150 followed by 1 h incubation at 37 °C with FITC-conjugated secondary antibody (JacksonImmuno). Nuclei were stained with DAPI. Samples were analyzed with a Leica DM 5000B microscope and Zeiss LSM 700 confocal microscope.

2.6. Data analysis

All data are expressed as the mean with standard deviation. Student's *t*-test was used to determine statistical significance of the results and p-values of < 0.05 were considered statistically significant. In addition, one-way ANOVA with a *post-hoc* Bonferroni multiple comparison test using GraphPad Prism4 software. A value of $p < 0.05$ was considered significant.

3. Results

A moderate and prolonged exercise training induces SIRT1 expression and activation in infarcted myocardium reducing the expression of pro-apoptotic genes.

Forty-eight hours post MI, exercised rats (EX) showed a significant increase of SIRT1 expression measured by western blot as compared to

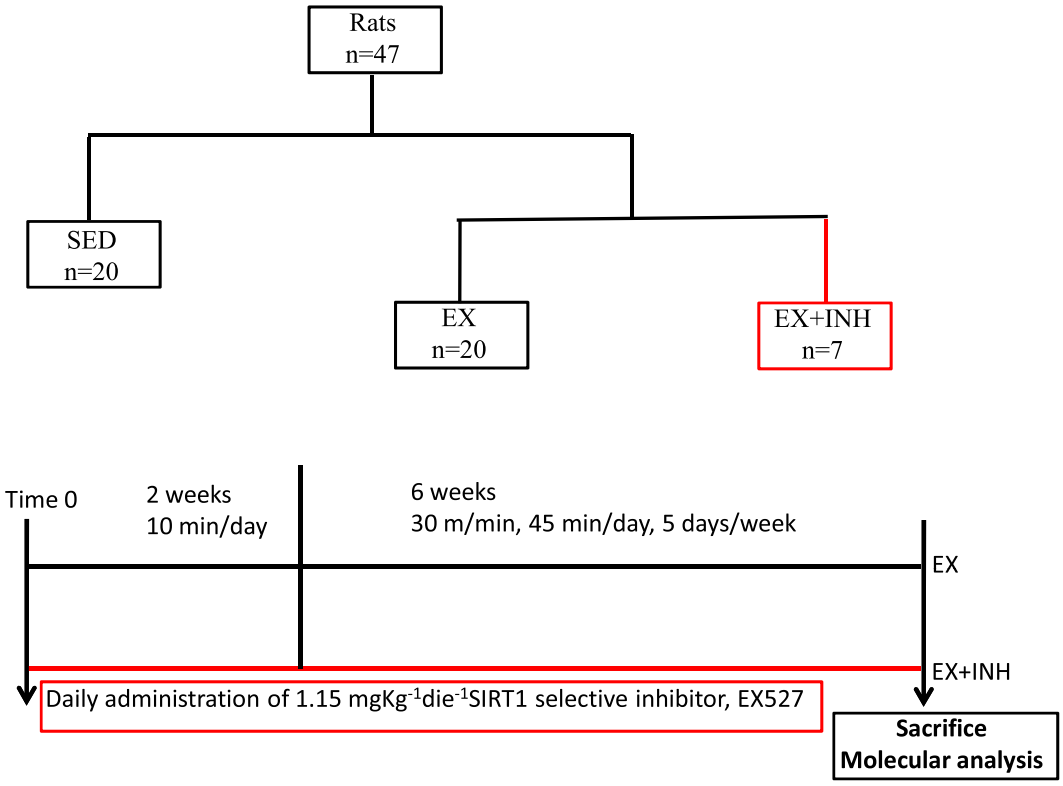

Fig. 1. Flow-chart describing the experimental plan.

the sedentary rats (SED). Compared to exercised infarcted rats, treatment with EX527, a competitive SIRT1 inhibitor, reduced significantly SIRT1 expression. This effect was more evident in the infarct border zone compared to the remote myocardium (Fig. 2B). To investigate whether exercise training exerts a cardioprotective effect early after MI through the activation of one of the SIRT1 pathway, we analyzed the expression of acetylated p53 which is a non-histonic target deacetylated by SIRT1. A prolonged and moderate exercise training caused a significant decrease of p53 acetylation compared to the sedentary group, indicating indirectly the enhanced activity of SIRT1. This was confirmed with the use of SIRT1 inhibitor EX527 that counteracted the effect of exercise training on the level of p53 acetylation. The magnitude of this effect was statistically significant only in the border zone (Fig. 2C). Acetylation of p53 enhances p53 binding to target genes, like Bax and caspase 3 promoting their transcription [14].

Therefore, we measured the expression of these two downstream pro-apoptotic genes regulated by p53. The expression of both proteins was reduced after exercise training and this effect was inhibited by EX527 administration. This result was less evident in the remote area of infarcted hearts (Fig. 2D and E).

A moderate and prolonged exercise training reduces cardiomyocytes apoptosis and promotes nuclear localization of SIRT1.

To determine whether moderate and prolonged exercise can benefit the infarcted myocardium, the hearts were analyzed forty-eight hours after coronary artery ligation. Typical features of early myocardial infarction were present in H&*E*-stained sections (data not shown). Apoptotic cardiomyocytes were detected with a TUNEL assay and confocal microscopy. Interestingly, when compared to sedentary infarcted rats, the fraction of apoptotic myocytes in the area bordering infarct was significantly reduced in exercised animals (Fig. 3A). The extent of myocyte apoptotic death in the remote myocardium was comparable between experimental groups. After treatment with EX527 a potential trend to reverse the positive effect of exercise was observed, although did not reach statistical significance (Fig. 3B).

Graph (C) Myocyte apoptotic index expressed as mean ± SD. SED: sedentary infarcted rats; EX: exercised infarcted rats; EX + INH: exercised infarcted rats treated with SIRT1 inhibitor. * $p < 0.001$ *vs* SED.

Furthermore, the percentage of myocyte nuclei positive for SIRT1 was higher in the border zone of the infarcted hearts from exercised rats as compared to sedentary animals (Fig. 4A). In the remote myocardium, the extent of nuclear localization of SIRT1 was lower than in the border zone and did not change after exercise nor the use of SIRT1 inhibitor (Fig. 4B). An increased number of cardiomyocytes with a nuclear localization of SIRT1 in exercised animals may reflect a different functional status of SIRT1 in myocardial cells of exercised animals inducing a protective, anti-apoptotic effect. In fact, in the myocardium of SIRT1-inhibitor treated rats, the fraction of apoptotic myocytes showed an increasing trend while the number of myocyte nuclei with SIRT1 immunolabeling exhibited an inverse, decreasing tendency (Fig. 4B). Although these parameters did not reach statistical significance, they are consistent with our molecular biology data and confirm the protective role of SIRT1 in the ischemia-induced cardiomyocyte injury [24].

3.1. Antioxidant effects of exercise training in infarcted rats involve SIRT3 activation

It is well known that oxidative stress plays a pivotal role in the ischemic condition. Several studies have shown that SIRT3 overexpression induced by exercise training protects cardiomyocytes from the oxidative stress [25]. To verify if similar effects were also present early after myocardial infarction that followed a prolonged and moderate exercise, we have measured cardiac SIRT3 expression. Exercise training caused a significant increase of SIRT3 expression compared to the sedentary rats. On the other hand, a treatment with EX527 caused a significant decrease of SIRT3 protein expression and this effect were more evident in the infarct border zone than in the remote myocardium (Fig. 5B).

Other targets that participate in sirtuin signalling are FOXOs transcription factors. In particular, FOXO-3a has a protective role in cardiomyocytes through the induction of antioxidant proteins expression

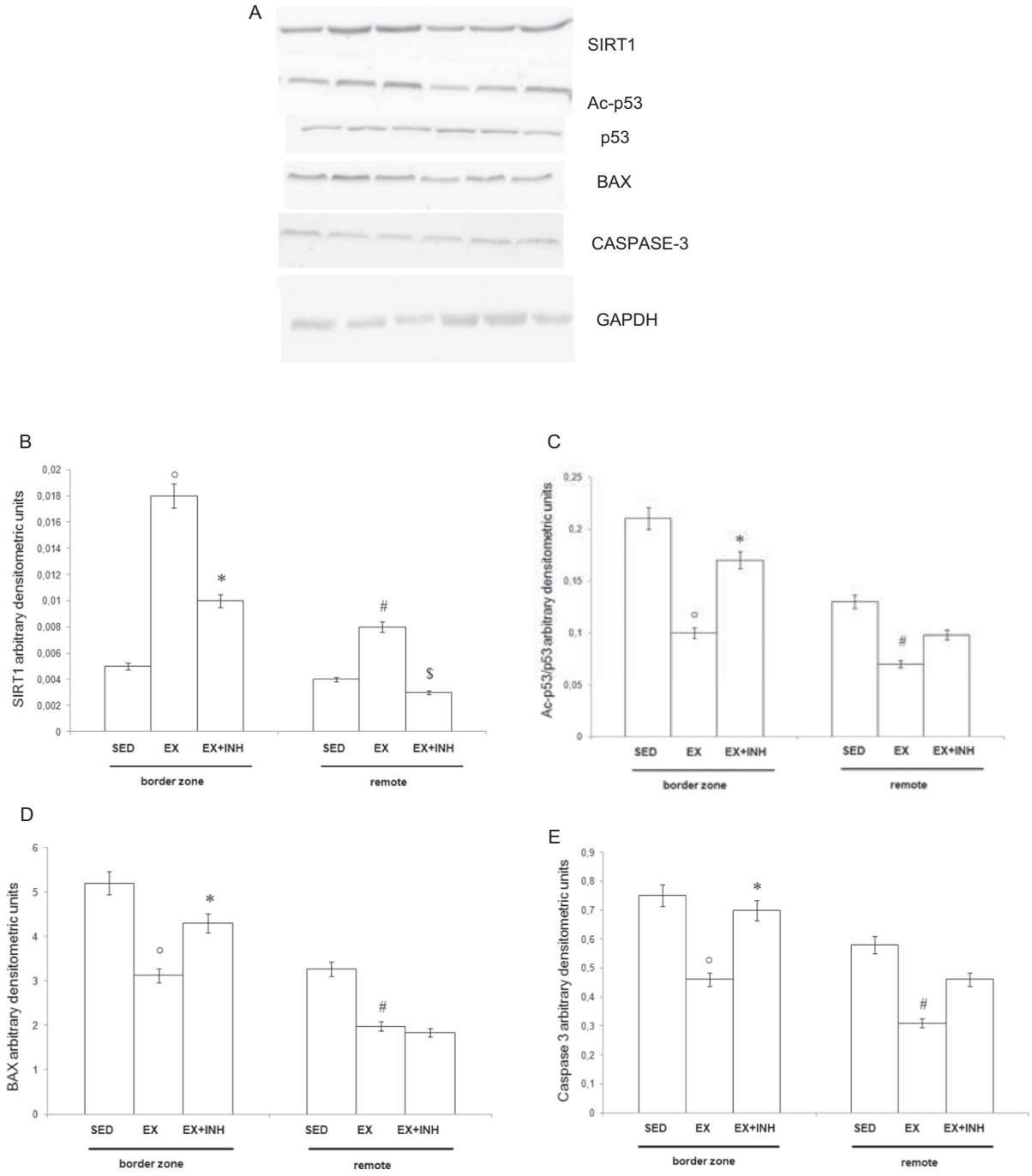

Fig. 2. Protective effects of exercise training on myocardial infarction through SIRT1 activation. Representative Western blot (B-E) and average densitometric quantitative analysis (A) from blots showing the ratio of SIRT1 (B), Ac-p53 (C), Bax (D) and caspase 3 (E) to GAPDH on infarcted cardiac muscle tissues in sedentary, trained and EX527 treated trained rats after myocardial infarction. Values were expressed in arbitrary densitometric units. Data are presented as mean ± SEM. ° $p < 0.05$ *vs* SED border zone; * $p < 0.05$ *vs* EX border zone; # $p < 0.05$ *vs* SED remote zone; $ $p < 0.05$ *vs* EX remote zone.

[17].

In comparison to the sedentary infarcted rats, a prolonged and moderate exercise training that preceded an acute ischemic injury, resulted in an increase of FoxO3a (Fig. 5C) and of its two downstream targets, MnSOD (Fig. 5D) and catalase (Fig. 5E). Moreover, a daily treatment with EX527 caused a significant decrease of FoxO-3a, MnSOD and catalase protein expression in the trained rats (Fig. 5C, D, E). These data indicate that exercise training prior to myocardial infarction positively modulates the SIRT3 pathway enhancing the expression of anti-oxidant proteins. Increased anti-oxidant defense can interfere with the activation and the progression of MI-induced oxidative damage that is aggravated by a sedentary state.

4. Discussion

The findings of this study demonstrate that a moderate and prolonged exercise training exerts a beneficial effect in the early phase of an acute myocardial infarction. In particular, postoperative mortality was significantly higher in the sedentary group than in trained or treated with SIRT1 inhibitor ones (25% *vs* 12 or 18%, respectively).

A regular physical activity represents an important therapeutic intervention for prevention and improvement of the outcome in

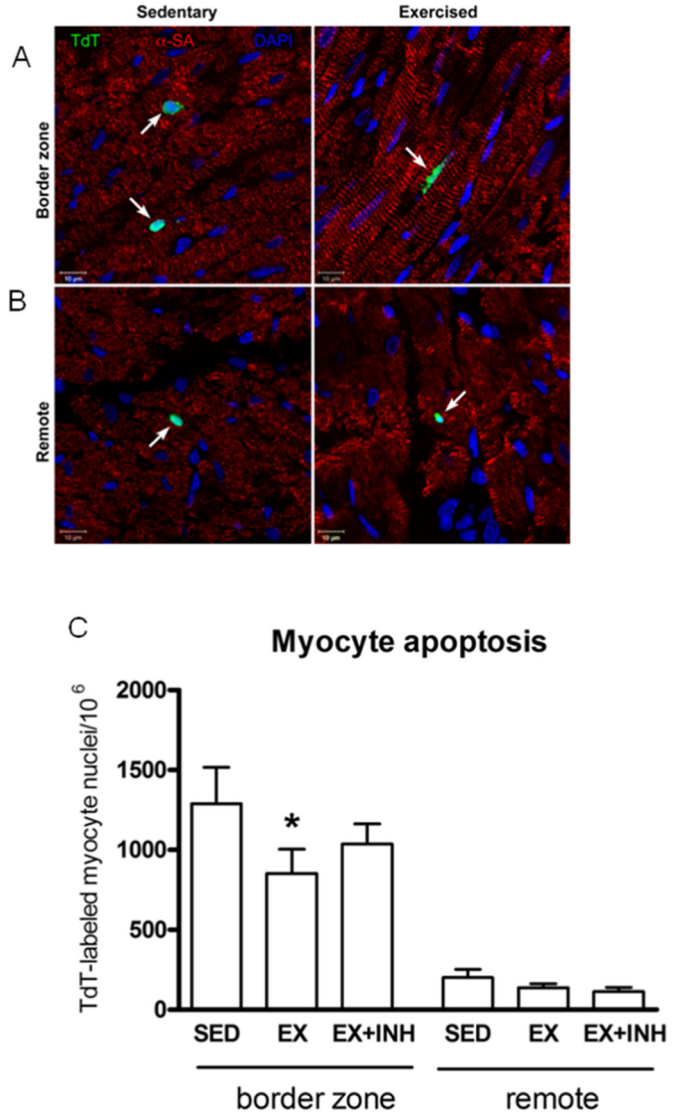

Fig. 3. A moderate and prolonged exercise training reduced the apoptotic signalling. Image: Examples of apoptosis (TdT, green, arrows) of cardiomyocytes labelled with α-sarcomeric actin (red) in the border zone (A) and remote (B) myocardium from sedentary and exercised infarcted rats. Nuclei are stained with DAPI (blue). Scale bars: 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

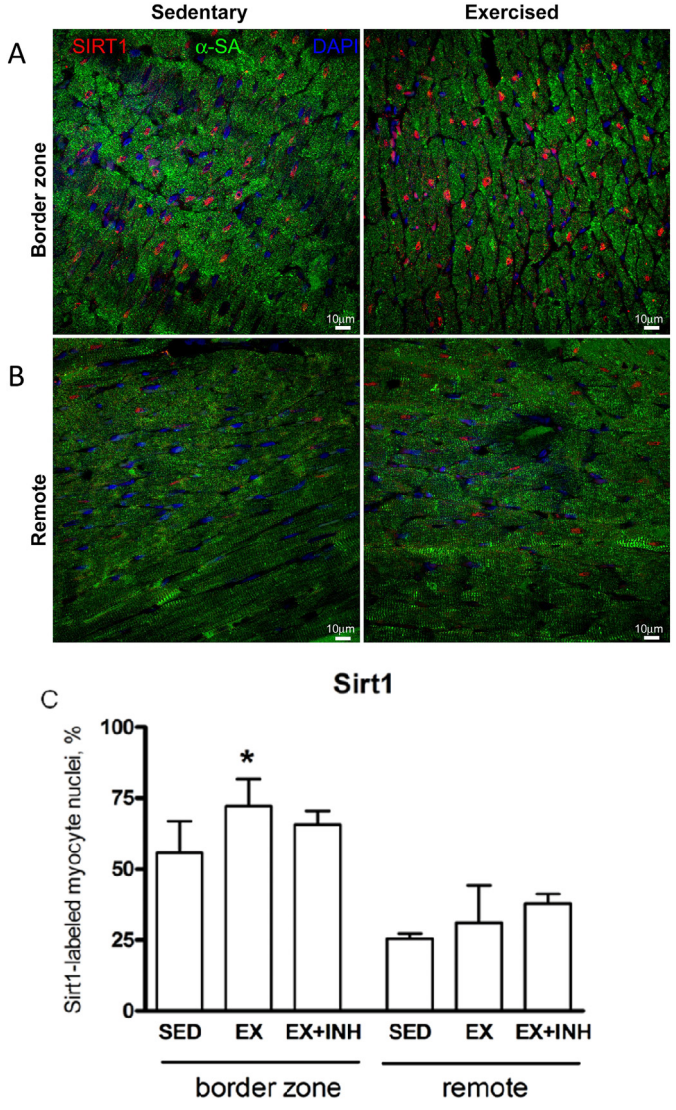

Fig. 4. Effect of exercise training on SIRT1 localization in cardiac infarcted heart. SIRT1 Image.
Nuclear localization of SIRT1 (red) in cardiomyocytes labelled with α-sarcomeric actin (green) in the border zone (A) and remote (B) myocardium from sedentary and exercised infarcted rats. Nuclei are stained with DAPI (blue). Scale bars: 10 μm.
Graph (C) The fraction of SIRT1-positive myocyte nuclei expressed as mean ± SD. SED: sedentary infracted rats; EX: exercised infracted rats; EX + INH: exercised infracted rats treated with SIRT1 inhibitor. * $p < 0.05$ *vs* SED.

cardiovascular diseases [5]. A proposed cardioprotective mechanism included a reduction of oxidative stress and cell death [26]. In this work we showed that our particular modality of training exerts an anti-apoptotic and antioxidant activity reducing early myocardial damage through the activation of SIRT1 and SIRT3 pathways.

Accumulating evidence suggest that the sirtuins are regulated by exercise, especially regarding activation of signalling pathways that require an enhanced SIRT1 activity [8,9].

It is well documented by *in vitro* and *in vivo* studies that SIRT1 is involved in the pathogenesis of cardiovascular diseases regulating a wide array of cellular processes [27,28]. SIRT1 has been shown to have a protective role against ischemic injury and in a non-ischemic model of heart failure [13,29].

Our result demonstrated that in infarcted rats, this model of training induced an increased cardiac expression of SIRT1 and that the SIRT1 inhibitor, EX527, counteracted significantly this effect prompting the ability of this inhibitor to interfere with SIRT1 pathway. Previous studies have highlighted the role of SIRT1 activation in the protective effects of exercise training, but there are few data about the pathway induced by exercise in the infarcted hearts [30]. Molecular data showed that within the infarcted myocardium of exercised rats, levels of acetyl-p53, a direct target of SIRT1, were significantly lower [31]. Therefore, we have chosen to analyze the pathways induced by acetylated p53. Exercise training induced a cardioprotective effect through the reduction of apoptosis; this effect was more evident in border zone than in remote portion and was accompanied by the reduction of p53 acetylation and decreased expression of its two downstream targets, Bax and caspase 3. The involvement of SIRT1 as an upstream inductor of this cascade was confirmed by the significant reduction of all these targets in the presence of EX527, an inhibitor of SIRT1 signalling. The changes in pro-apoptotic molecular regulators were reflected in the quantitative data on cardiomyocyte apoptotic death rate that was

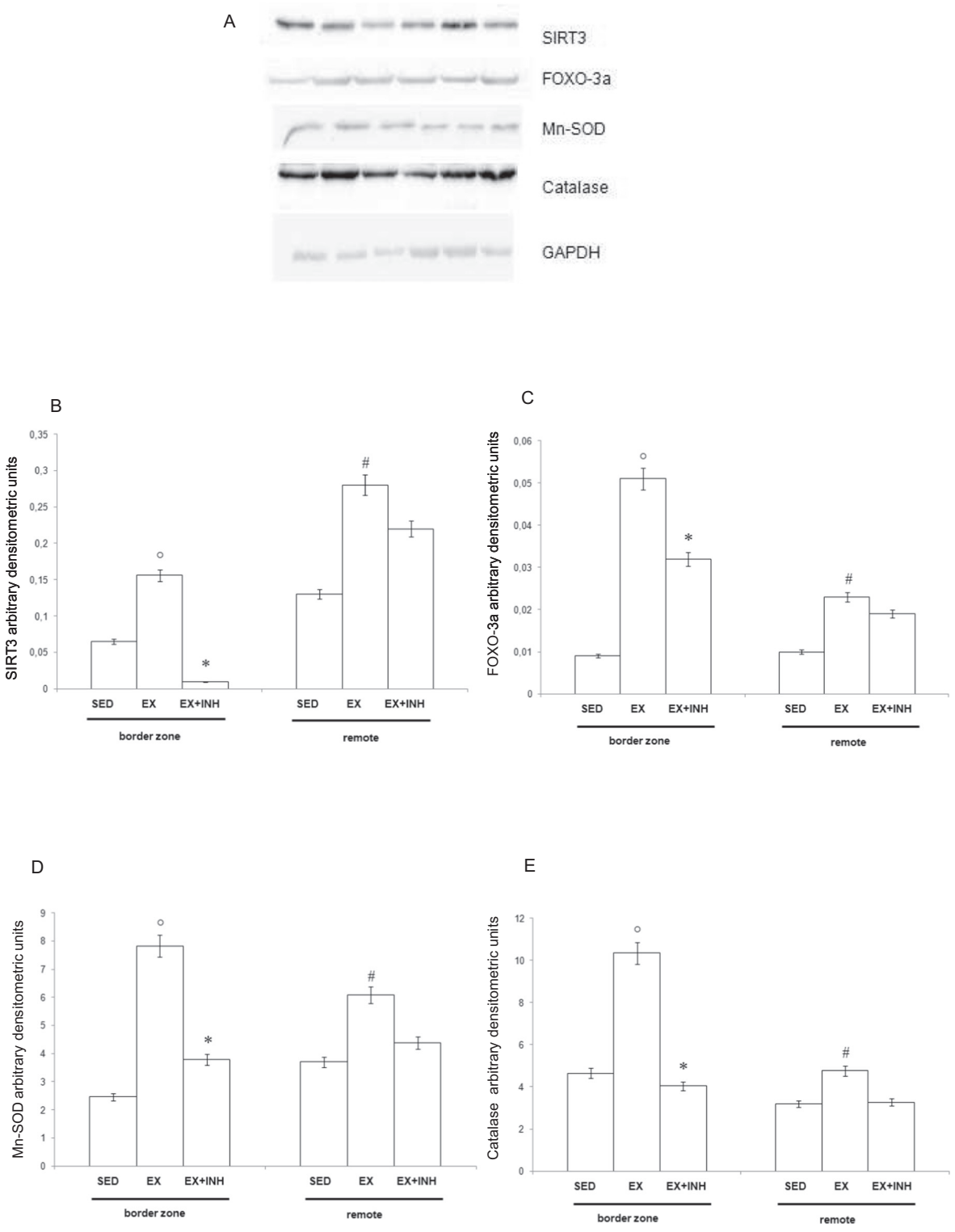

Fig. 5. SIRT3 pathway activation induced by exercise training reduces oxidative stress in myocardial infarction. Representative Western blot (B-E) and average densitometric quantitative analysis (A) from blots showing the ratio of SIRT3 (B), FOXO-3a (C), MnSOD (D) and Catalase (E) to GAPDH on infarcted cardiac muscle tissues in sedentary, trained and EX527 treated trained rats. Values are expressed in arbitrary densitometric units. Data are presented as mean ± SEM. ° $p < 0.05$ *vs* SED border zone; * $p < 0.05$ *vs* ex border zone; # $p < 0.05$ *vs* SED remote zone.

significantly decreased in exercised rats compared to sedentary animals, strengthening the concept that physical activity prior to ischemic insult can have protective effects against cardiomyocyte apoptotic death in area bordering myocardial infarction. The results observed in the peri-infarct region may be of clinical relevance as the myocardial tissue adjacent to the area of maximal ischemia is considered to be in a state of reversible injury and participates in the sustaining of the ventricular function.

Although SIRT1 has classically been considered a nuclear protein, it has also been proposed that the cytoplasmic-nuclear shuttling of SIRT1 can be correlated with its protective effects increasing the ability to deacetylate nuclear targets [32]. In this regard, an increased number of

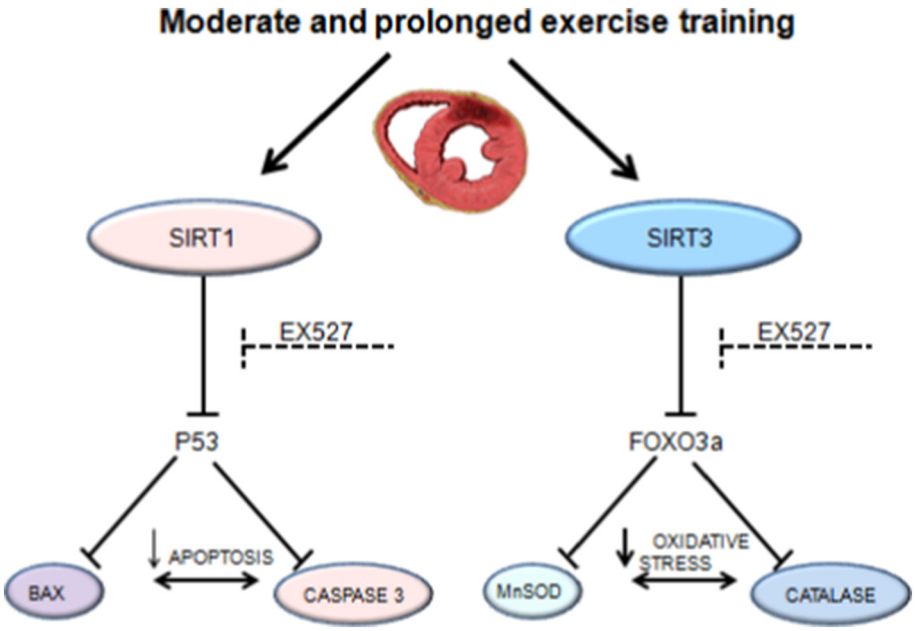

Fig. 6. The potential cardioprotective effect of exercise training. Our model of a moderate and prolonged exercise training induced SIRT1-p53 and SIRT3-FoxO3a-dependent pathway reducing apoptosis and oxidative stress.

cardiomyocytes with a nuclear localization of SIRT1 in exercised animals may reflect a different functional status of SIRT1 in myocardial cells of exercised animals. In fact, our molecular data shown that within the infarcted myocardium of exercised rats levels of acetyl-p53 were significantly lower. Moreover, the expression of several SIRT1-regulated proteins was also affected and all these results are clearly counteracted by the treatment with SIRT inhibitor. Our results indicate that the activation of SIRT1 was implicated in the reduction of apoptosis, that is one of several cardiac beneficial effects of moderate exercise.

Increasing evidence suggests a role of SIRT3 in cardiac diseases such as heart failure, cardiac hypertrophy and myocardial infarction. This deacetylase is highly expressed in the heart where it has a protective role in maintaining cell homeostasis, particularly in stress condition [33].

In particular, it has been shown that the hearts of SIRT3 deficient mice are more exposed to cardiovascular oxidative stress and the lack of this deacetylase increased the extension of cardiac ischemic area after ischemia-reperfusion [19]. SIRT3 is able to prevent the accumulation of ROS by binding the promoter of anti-oxidant genes [34]. For this purpose, we have investigated another cardioprotective effect of exercise training that is the reduction of oxidative stress. To this end we have measured SIRT3 expression and we found that exercise training increased SIRT3 levels in infarcted hearts. Before to evaluate SIRT3-dependent oxidative pathway we tested the effects of the inhibitor EX527 on cardiac SIRT3 expression; our results showed that EX527 reduced SIRT3 expression induced by exercise with a statistical significance only in border zone. Multiple cellular pathways are regulated by SIRT3; in particular, one of stress responsive *via* includes activation of two FOXO-3a-dependent antioxidant enzymes, manganese superoxide dismutase (MnSOD) and catalase [35]. Our data showed that exercise training induced an activation of FOXO-3a pathway with an increased expression of MnSOD and catalase. This effect, more evident in the border zone, was counteracted by the inhibitor EX527 confirming an involvement of SIRT3 in the antioxidant effects of exercise training. We need to acknowledge the limitation, that while histological and molecular biology data obtained in the EX527-treated rats are strongly indicative, for economical reasons, the number of infarcted animals that received SIRT inhibitor was likely too low to reach statistical significance.

5. Conclusion

In conclusion, we have confirmed a role of a moderate and prolonged exercise training as a non-pharmacological approach in the prevention of cardiovascular diseases. We have investigated molecular mechanisms that underlined this effect and we found that the exercise-induced cardioprotection consists of the reduction of hypoxic damage and involves the activation of sirtuin's pathway (Fig. 6). Overall, our data support the concept of an exercise as a particular form of preconditioning [36], and show its ability to attenuate the early detrimental effects of myocardial ischemia. While long-term cellular and molecular effects of moderate prolonged training remain to be investigated, our data might also suggest novel targets for pharmacological modulation with a clear translational potential.

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Author contribution

Maria Donniacuo performed the experiments and wrote the paper; Konrad Urbanek participated in experimental design and in the revision of the manuscript; Angela Nebbioso provided assistance in data analysis and methodology; Loredana Sodano performed and analyzed the experiments; Laura Gallo performed experiments; Lucia Altucci participated in drafting of the manuscript; Barbara Rinaldi provided funding, conceived the research and revised the manuscript.

All authors read and approved the final manuscript.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

[1] G.W. Reed, J.E. Rossi, C.P. Cannon, Acute myocardial infarction, Lancet 389 (2017) 197–210, https://doi.org/10.1016/S0140-6736(16)30677-8.

[2] G.W. Moe, J. Marín-García, Role of cell death in the progression of heart failure, Heart Fail. Rev. 21 (2016) 157–167, https://doi.org/10.1007/s10741-016-9532-0.
[3] J.G. Farías, V.M. Molina, R.A. Carrasco, A.B. Zepeda, E. Figueroa, P. Letelier et al., 2017. Antioxidant therapeutic strategies for cardiovascular conditions associated with oxidative stress, Nutrients 9, E966. doi:https://doi.org/10.3390/nu9090966.
[4] T.M. Eijsvogels, S. Molossi, D.C. Lee, M.S. Emery, P.D. Thompson, Exercise at the extremes: the amount of exercise to reduce cardiovascular events, Am. Coll. Cardiol. 67 (2016) 316–329, https://doi.org/10.1016/j.jacc.2015.11.034.
[5] L. Tao, Y. Bei, S. Lin, H. Zhang, Y. Zhou, J. Jiang et al., Exercise training protects against acute myocardial infarction via improving myocardial energy metabolism and mitochondrial biogenesis, Cell. Physiol. Biochem. 37 (2015) 162–175. doi:https://doi.org/10.1159/000430342.
[6] J.K. Lew, J.T. Pearson, D.O. Schwenke, R. Katare, Exercise mediated protection of diabetic heart through modulation of microRNA mediated molecular pathways, Cardiovasc. Diabetol. 16 (2017) 10, , https://doi.org/10.1186/s12933-016-0484-4.
[7] G. Russomanno, G. Corbi, V. Manzo, N. Ferrara, G. Rengo, A.A. Puca et al., 2017. The anti-ageing molecule sirt1 mediates beneficial effects of cardiac rehabilitation, Immun. Ageing 14:7. doi:https://doi.org/10.1186/s12979-017-0088-1.
[8] N. Ferrara, B. Rinaldi, G. Corbi, V. Conti, P. Stiuso, S. Boccuti et al., Exercise training promotes SIRT1 activity in aged rats, Rejuvenation Res.. 11 (2008) 139–150. doi:https://doi.org/10.1089/rej.2007.0576.
[9] V. Carafa, D. Rotili, M. Forgione, F. Cuomo, E. Serretiello, G.S. Hailu et al., 2016. Sirtuin functions and modulation: from chemistry to the clinic, Clin. Epigenetics, 8:61. doi:https://doi.org/10.1186/s13148-016-0224-3.
[10] B. Sosnowska, M. Mazidi, P. Penson, A. Gluba-Brzózka, J. Rysz, M. Banach, The sirtuin family members SIRT1, SIRT3 and SIRT6: their role in vascular biology and atherogenesis, Atherosclerosis 265 (2017) 275–282. doi:https://doi.org/10.1016/j.atherosclerosis.2017.08.027.
[11] X. Meng, J. Tan, M. Li, S. Song, Y. Miao, Q. Zhang, Sirt1: role under the condition of ischemia/hypoxia, Cell. Mol. Neurobiol. 37 (2017) 17–28, https://doi.org/10.1007/s10571-016-0355-2.
[12] S. Winnik, J. Auwerx, D.A. Sinclair, C.M. Matter, Protective effects of sirtuins in cardiovascular diseases: from bench to bedside, Eur. Heart J. 36 (2015) 3404–3412, https://doi.org/10.1093/eurheartj/ehv290.
[13] L. Wang, N. Quan, W. Sun, X. Chen, C. Cates, T. Rousselle, X. Zhou, X. Zhao, J. Li, Cardiomyocyte- specific deletion of Sirt1 gene sensitizes myocardium to ischemia and reperfusion injury, Cardiovasc. Res. 114 (2018) 805–821, https://doi.org/10.1093/cvr/cvy033.
[14] X.L. Wang, L.Y. Wu, L. Zhao, L.N. Sun, H.Y. Liu, G. Liu, G.J. Guan, SIRT1 activator ameliorates the renal tubular injury induced by hyperglycemia in vivo and in vitro via inhibiting apoptosis, Biomed. Pharmacother. 83 (2016) 41–50, https://doi.org/10.1016/j.biopha.2016.06.009.
[15] A. Brunet, L.B. Sweeney, J.F. Sturgill, K.F. Chua, P.L. Greer, Y. Lin et al., Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase, Science 303 (2004) 2011–2015. doi:https://doi.org/10.1126/science.1094637.
[16] A. Peserico, F. Chiacchiera, V. Grossi, A. Matrone, D. Latorre, M. Simonatto et al., A novel AMPK-dependent FoxO3A-SIRT3 intramitochondrial complex sensing glucose levels, Cell. Mol. Life Sci. 70 (2013) 2015–2029. doi:https://doi.org/10.1007/s00018-012-1244-6.
[17] P. Rangarajan, A. Karthikeyan, J. Lu, E.A. Ling, S.T. Dheen, Sirtuin 3 regulates Foxo3a-mediated antioxidant pathway in microglia, Neurosci 311 (2015) 398–414, https://doi.org/10.1016/j.neuroscience.2015.10.048.
[18] C. Koentges, C. Bode, H. Bugger, SIRT3 in cardiac physiology and disease, Front. Cardiovasc. Med. 3 (2016) 38, https://doi.org/10.3389/fcvm.2016.00038.
[19] W. Yu, B. Gao, N. Li, J. Wang, C. Qiu, G. Zhang et al., Sirt3 deficiency exacerbates diabetic cardiac dysfunction: role of Foxo3A-Parkin-mediated mitophagy, Biochim. Biophys. Acta 1863 (2017) 1973–1983. doi:https://doi.org/10.1016/j.bbadis.2016.10.021.
[20] B. Rinaldi, M. Donniacuo, L. Sodano, G. Gritti, S. Signoriello, E. Parretta et al., 2013. Effects of sildenafil on the gastrocnemius and cardiac muscles of rats in a model of prolonged moderate exercise training, PLoS One 8: e69954. doi:https://doi.org/10.1371/journal.pone.0069954.
[21] B. Rinaldi, F. Guida, A. Furiano, M. Donniacuo, L. Luongo, G. Gritti et al., 2015. Effect of prolonged moderate exercise on the changes of nonneuronal cells in early myocardial infarction. Neural. Plast. 2015: 265967. doi:https://doi.org/10.1155/2015/265967.
[22] B. Rinaldi, M. Donniacuo, L. Sodano, G. Gritti, E. Martuscelli, A. Orlandi et al., Effects of chronic treatment with the new ultra-long-acting β2 -adrenoceptor agonist indacaterol alone or in combination with the β1 -adrenoceptor blocker metoprolol on cardiac remodelling, Br. J. Pharmacol., 172 (2015) 3627–3637. doi:https://doi.org/10.1111/bph.13148.
[23] N.J. Kruger, The Bradford method for protein quantitation, Methods Mol. Biol. 32 (1994) 9–15, https://doi.org/10.1385/0-89603-268-X:9.
[24] Y. Guo, L. Zhang, F. Li, C.P. Hu, Z. Zhang, Restoration of sirt1 function by pterostilbene attenuates hypoxia-reoxygenation injury in cardiomyocytes, Eur. J. Pharmacol. 776 (2016) 26–33, https://doi.org/10.1016/j.ejphar.2016.02.052.
[25] H.K. Jiang, Y. Miao, Y.H. Wang, M. Zhao, Z.H. Feng, X.J. Yu et al., Aerobic interval training protects against myocardial infarction-induced oxidative injury by enhancing antioxidase system and mitochondrial biosynthesis, Clin. Exp. Pharmacol. Physiol. 41 (2014) 192–201. doi:https://doi.org/10.1111/1440-1681.12211.
[26] T. Brioche, S. Lemoine-Morel, Oxidative stress, sarcopenia, antioxidant strategies and exercise: molecular aspects, Curr. Pharm. Des. 22 (2016), https://doi.org/10.2174/1381612822666160219120531 (2664–2278).
[27] C. Granchi, F. Minutolo, Activators of sirtuin-1 and their involvement in cardioprotection, Curr. Med. Chem. (2018), https://doi.org/10.2174/0929867325666180214115438.
[28] D. Cappetta, G. Esposito, E. Piegari, R. Russo, L.P. Ciuffreda, A. Rivellino et al., SIRT1 activation attenuates diastolic dysfunction by reducing cardiac fibrosis in a model of anthracycline cardiomyopathy, Int. J. Cardiol. 205 (2016) 99–110. doi:https://doi.org/10.1016/j.ijcard.2015.12.008.
[29] A. De Angelis, E. Piegari, D. Cappetta, R. Russo, G. Esposito, L.P. Ciuffreda et al., SIRT1 activation rescues doxorubicin-induced loss of functional competence of human cardiac progenitor cells, Int. J. Cardiol. 189 (2015) 30–44. doi:https://doi.org/10.1016/j.ijcard.2015.03.438.
[30] S.H. Chan, C.H. Hung, J.Y. Shih, P.M. Chu, Y.H. Cheng, H.C. Lin et al., Exercise intervention attenuates hyperhomocysteinemia-induced aortic endothelial oxidative injury by regulating SIRT1 through mitigating NADPH oxidase/LOX-1signaling, Redox Biol., 14 (2018) 116–125. doi:https://doi.org/10.1016/j.redox.2017.08.016.
[31] W. Hong, S. Tatsuo, W. Shou-Dong, Z. Qian, H. Jian-Feng, W. Jue et al., 2015. Resveratrol upregulates cardiac SDF-1 in mice with acute myocardial infarction through the deacetylation of cardiac p53, PLoS One, 10: e0128978. doi:https://doi.org/10.1371/journal.pone.0128978.
[32] S. Yanagisawa, J.R. Baker, C. Vuppusetty, T. Koga, T. Colley, P. Fenwick et al., 2018. The dynamic shuttling of SIRT1 between cytoplasm and nuclei in bronchial epithelial cells by single and repeated cigarette smoke exposure, PLoS One, 13: e0193921. doi:https://doi.org/10.1371/journal.pone.0193921.
[33] Y. Lu, Y.D. Wang, X.Y. Wang, H. Chen, Z.J. Cai, M.X. Xiang, SIRT3 in cardiovascular diseases: emerging roles and therapeutic implications, Int. J. Cardiol. 220 (2016) 700–705, https://doi.org/10.1016/j.ijcard.2016.06.236.
[34] J. Liu, D. Li, T. Zhang, Q. Tong, R.D. Ye, L. Lin, SIRT3 protects hepatocytes from oxidative injury by enhancing ROS scavenging and mitochondrial integrity, Cell Death Dis. 8 (2017) e3158, , https://doi.org/10.1038/cddis.2017.564.
[35] P. Rangarajan, A. Karthikeyan, J. Lu, E.A. Ling, S.T. Dheen, Sirtuin 3 regulates Foxo3a-mediated antioxidant pathway in microglia, Neuroscience 311 (2015) 398–414, https://doi.org/10.1016/j.neuroscience.2015.10.048.
[36] D.H.J. Thijssen, A. Redington, K.P. George, M.T.E. Hopman, H. Jones, Association of exercise preconditioning with immediate cardioprotection: a review, JAMA Cardiol. 3 (2018) 169–176, https://doi.org/10.1001/jamacardio.2017.4495.