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Role of sirtuins, calorie restriction and physical activity in aging

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1. ABSTRACT

Recently it has been discovered that Sirtuins represent pivotal regulators of lifespan. Caloric restriction (CR) enhances longevity from yeast to mammals. Whereas the relationship between Sirt-1 and CR is clear, the molecular mechanisms by which Sir2 increases longevity are still unknown. In mammals, CR induces physiological and behavioral changes, and many studies have shown that CR decreases production of reactive oxygen species production thus minimizing oxidative damage, leading to the hypothesis that CR by reducing oxidative stress extends the lifespan by counteraction of aging. In fact, the pathophysiology of aging and age-related diseases involves oxidative stress as an early stage in its development. Recently we found that in aged rats the SIRT1 activity was decreased in heart and adipose tissue, showing as aging is characterized *in vivo* by a reduced efficiency of this key-regulator of longevity. Whereas several studies have reported that increased physical activity can improve mean life span presumably by reducing mortality risk from many age-related diseases, exercise and longevity studies have failed to document an exercise effect on maximum life span. However, in aged rats a moderate prolonged exercise training is able to induce increase in SIRT1 activity, suggesting that this tool could counteract age-related dysfunctions.

2. INTRODUCTION

In the last decades the worldwide population has exhibited an increasing life expectancy with a consequent raise in elderly population. Whereas the reason of this phenomenon could be related to an improvement in medical treatment and disease's prevention, the mechanisms underlying longevity are only partially known. The genetic hypothesis is able to explain only 20 to 30% of the longevity in humans (1-3). In fact although all species have a defined life-span, simple modifications of the environment, including caloric restriction or sublethal levels of stress, can substantially affect organismal longevity. The ageing phenotype is a complex interaction of stochastic, environmental, genetic and epigenetic variables. However, these variables do not create the ageing phenotype but generate the lost of molecular fidelity and therefore as the random accumulation of damage in the human organism's cells, tissues, or whole organism during life increases, the probability of disease and death also augments in proportion. Indeed aging is accompanied by a decline in the healthy function of multiple organ systems, leading to increased incidence and mortality from diseases such as type II diabetes mellitus, neurodegenerative diseases, cancer, and cardiovascular disease.

In fact, ageing, senescence and death are the final manifestations of unsuccessful homeostasis or failure of homeodynamics. A progressive shrinking of the homeodynamic property is the hallmark of aging and the cause of origin of age-related diseases. (4)

Recently it has been discovered that a family of enzymes consists of NAD+-dependent histone/protein deacetylases, called Sirtuins, represents pivotal regulator of longevity and healthspan. In particular overexpression of Sir2 (silencing information regulator 2), the first gene of this family discovered in yeast, has been demonstrated to extend the life span in various organisms. The anti-aging effects of human homologues of sirtuins, SIRT1-7, have also been suggested by animal and human association studies. However, the precise mechanisms whereby sirtuins exert their anti-aging effects remain elusive.

3. CLASSES AND FUNCTIONS OF SIRTUINS

In mammals seven homologues of Sir2 gene have been identified to date. SIRT1, evolutionarily the closest protein to the yeast Sir2, has been found in both nucleus and cytoplasm (5,6), and trough the deacetylation of histones and other cellular factors such as p53, NFkB, HSF1, FOXOs and PGC-1, it is able to regulate cell's biology, metabolism and fate at different levels (7).

SIRT2 is highly expressed in heart, brain, testis, and skeletal muscle (8, 9). The protein is cytoplasmic (8-10), but has been found also in the nucleus, and its levels are regulated during the cell cycle. SIRT2 increases dramatically during mitosis, and it becomes phosphorylated at the G2/M transition, and its overexpression dramatically prolongs the M phase. Moreover, SIRT2 is targeted for degradation by the proteasome (11).

SIRT3 gene is highly expressed in brain, heart, liver, kidney, testis, and muscle (8, 12), and the Sirt3 protein is localized to the mitochondrial matrix (13). Biological targets of this protein have to be better identified.

SIRT4 is another member of the Sir2 family, broadly expressed in all tissues, except leukocytes in the adult and thymus in the fetus (14). SIRT4 may be enzymatically inactive as histone deacetylase *in vitro* (10), and its biological role has not been yet completely understood. Both SIRT3 and SIRT4, with a coordinate activity, seems to inhibit cell death by maintaining mitochondrial NAD+ levels after a stress challenge.

SIRT5 gene is broadly expressed (14), it is a mitochondrial protein enzymatically active as a histone deacetylase (10), and its biological significance has to be discovered.

SIRT6 is a nuclear protein and recently it has been shown to act as a histone deacetylase that can influence the telomers of human cells (15). SIRT6 deficient cells demonstrated increased genomic instability and sensitivity to DNA damage, and Sirt6 has been also shown to antagonize cell senescence in human keratinocytes, probably through the regulation of NFkB activity (16).

SIRT7 is a nuclear protein, highly expressed in the spleen, ovary, and thyroid. SIRT7 is also highly expressed in thyroid carcinomas when compared to normal thyroid tissues. In contrast, its expression is almost undetectable in adenomas and normal thyroid tissues (17, 18). Its activity has been associated to cardiovascular physiology, as SIRT7-deficient mice exhibit cardiac abnormalities. SIRT7 is enzymatically inactive as a histone deacetylase (10).

Although all the seven sirtuins are probably equally important, most of the studies conducted to unveil the mechanisms of action and biological relevance of these pleiotropic proteins have involved only SIRT1. Thus, in the recent years, accumulating data have shown that SIRT1 represents a critical upstream enzyme able to regulate fundamental cell biological processes including gene expression, genome stability, mitosis, nutrient metabolism, mitochondrial function and ageing (19).

4. ROLE OF SIRTUINS IN THE CELLULAR STRESS RESPONSE

Brunet *et al.* have shown that in mammalian cells SIRT1 appears to control the cellular response to stress by regulating the family of Forkhead transcriptional factors (FOXOs), a family of proteins that function as sensors of the insulin signaling pathway and as regulators of organismal longevity. SIRT1 and the transcriptional factor FOXO3 form a complex in cells in response to oxidative stress, and SIRT1 deacetylates FOXO3 *in vitro* and within cells. SIRT1 has a dual effect on FOXO3 function, increasing FOXO3's ability to induce cell cycle arrest and resistance to oxidative stress but inhibiting its capacity to induce cell death. Thus, one way in which members of the Sir2 family of proteins may increase organismal longevity is by tipping FOXO-dependent responses away from apoptosis and toward stress resistance, by inhibition of apoptosis and promotion of DNA repair (20-23).

In addition, a recent study has demonstrated that SIRT1 can modulate the cellular stress response directly deacetylating the heat shock factor (HSF1) and thus regulating heat shock proteins (HSPs) expression (24). The cellular protection of HSPs is attributed to their molecular chaperone function by facilitating nascent protein folding and refolding or degradation of abnormally folded proteins. Cellular ability to maintain adequate expression levels of protective genes such as HSP70, in response to a stressful insult, such as free radicals exposure, seems to be essential to preserve cellular homeostasis, and to delay aging related degenerative processes (25). Inhibiting SIRT1 expression via small interfering RNA prevents HSF1 from binding to the *hsp70* promoter and suppresses transcription of the gene when cells are exposed to heat shock. On the contrary, SIRT1 activation by agents such as resveratrol, or SIRT1 overexpression in cells decreases HSF1 acetylation, prolongs HSF1 binding to target promoters, and enhances the heat shock response (24).

Moreover, SIRT1 modulates the threshold for cell death in the setting of exogenous stress by its ability to interact with p53. It has been shown to deacetylate the p53 tumor suppressor protein (5, 6, 26, 27, 28), which downregulates p53 via effects on stability and activity (29). Since deacetylation of p53 correlates with a decreased p53 transcriptional function, it is conceivable that sirtuin inhibition could lead to improved tumor suppression (30). Recently the relevance of this pathway has been enriched by the evidence that p53 itself can regulate SIRT1 expression through various means that include p53-dependent microRNAs (31). Besides these major mechanisms other evidences suggest a more complex involvement for SIRT1 in the regulation of cellular stress response. In fact, SIRT1 has been shown to inhibit Bax-induced apoptosis by deacetylating Ku70 (32), and to regulate other targets linked to cell death, such as E2F1 (33) and TGF-b signaling (34).

Recently it has also been shown that SIRT1, similarly to other stress proteins, is regulated at posttranscriptional level by the RNA binding protein HuR, that under stress situation, stabilizes the SIRT1 mRNA, and increases SIRT1 expression levels (35) .

As increased stress resistance frequently correlates with longevity in model organisms (36), the ability of SIRT1 to modulate stress resistance in mammalian cells suggests a potential link with mammalian aging. Cellular senescence, which can be induced by several stimuli, consists of a state of permanent cell cycle arrest associated with characteristic changes in cell morphology. Human and mouse fibroblasts undergo to a limited number of divisions in culture, eventually entering a state of cellular senescence known as replicative senescence (37, 38). Cellular senescence can be activated by various types of stressful stimuli, including telomere shortening or other forms of genotoxic stress eliciting DNA damage response mediated primarily by the p53 tumor suppressor pathway. In this context SIRT1 appears as a critical enzyme in the molecular cascade determining the decision of a cell to live or to die in a stress situation.

5. ROLE OF SIRTUINS IN CALORIC RESTRICTION

Caloric restriction (CR), defined as a reduction in organism energy intake, has been shown to enhance longevity from yeast to mammals. In particular CR reduces metabolic rate and oxidative damage, improves markers of diabetes such as insulin sensitivity, showing a decreased incidence of cardiovascular disease and effects on neuroendocrine and sympathetic nervous system in laboratory animals and some of these findings are being replicated now in ongoing human studies (39). In mammals, calorie restriction induces a complex pattern of physiological and behavioral changes, such as reduction in blood glucose, triglycerides, and growth factors (40 - 43).

It has been widely demonstrated that CR effects are mediated by Sir2. In particular, increased Sir2 activity leads to replicative life-span extension, but the link between Sir2 and CR remains unclear. Deletion of Sir2 in yeast abolishes the increase in life-span induced by caloric restriction or sublethal levels of stress, indicating that Sir2 is a mediator of signals that promote longevity (44, 45). Moreover, an increased dosage of Sir2 proteins extends life span, while deletion or mutation of Sir2 reaches the opposite result in several different species (28, 46 - 50). Whereas it is not yet known whether the mammalian Sir2 ortholog, Sirt1, similarly regulates aging and longevity in mammals, it has been shown to regulate metabolic responses to changes in nutrient availability in several tissues (51, 52), increasing in muscle, brain, liver, and fat in response to fasting and CR in rodents (32, 53 - 55). Up-regulation of Sirt1 in adipocytes in response to fasting promotes lipolysis and free fatty acid mobilization through repression of PPARγ, a nuclear hormone receptor that promotes adipogenesis (56). Sirt1 also promotes gluconeogenesis and represses glycolysis in hepatocytes in response to nutrient deprivation by interacting with and deacetylating PGC-1α, a key transcriptional regulator of glucose production in the liver (55). In skeletal muscle, Sirt1-mediated PGC-1α deacetylation is required to induce mitochondrial fatty acid oxidation genes in states of nutrient deprivation (57). Finally, Ramsey (58) and others demonstrated that Sirt1 promotes insulin secretion in pancreatic β cells in response to glucose (59, 60). Moreover the dependency of Sir2 on NAD potentially links the activation of Sir2 to metabolic changes that occur during glucose (caloric) restriction in yeast. These induce a switch from fermentation to respiration, which causes an increase in the NAD/NADH ratio (61). Many studies have also shown that CR decreases reactive oxygen species (ROS) production thus minimizing oxidative damage (62, 63), leading to the hypothesis that CR by reducing oxidative stress extends the lifespan. It has been proposed that life span is inversely related to the degree of membrane phospholipid unsaturation (64, 65) and that elucidation of this relationship can provide insight on the mechanism for life span extension with CR (66). Modulation of membrane susceptibility to peroxidation, however, may be too simplistic to explain aging processes with this hypothesis, that does not consider other processes, such as changes in cellular signaling, leakage of ions (67), ROS production (68), induction of apoptosis (69), and maintenance of adaptive antioxidant systems (39, 70 - 73).

6. ROLE OF SIRTUINS DURING AGING AND THEIR RELATION WITH PHYSICAL ACTIVITY

Aging is one of the greatest risk factors for metabolic complications, such as obesity, glucose intolerance, and type 2 diabetes (74, 75).

Moreover aging is also characterized by increasing prevalence of cardiovascular diseases, related to pathophysiological changes distinguishing “senile” heart. In particular, in the elderly, cardiac apoptosis and necrosis, proliferation of myocyte nuclei, increased myocyte volume, and connective tissue accumulation are observed (76 - 78). Another marker of “aged heart” is represented by attenuated induction of cell-protective mechanisms, such as antioxidants and heat shock proteins, in response to pathologic insults (79). In fact, the pathophysiology of aging and age-related diseases involves oxidative stress as an early stage in its development (80) as confirmed by a decrease in antioxidant defenses and an increase in oxidative damage (81, 82).

Some authors showed that Sirt1 provides protection against apoptosis and plays an essential role in mediating survival of cardiac myocytes and neurons under stress *in vitro* (83 - 85).

Recently, we found that in aged rats (24 months old) in comparison with young rats (6 months old), the SIRT1 activity was decreased in cardiac left ventricle and adipose tissue, showing that aging is characterized *in vivo* by a reduced efficiency of this key-regulator of longevity in rats (86). The sirtuins also appear to have a prominent role in vascular biology, and may regulate aspects of age-dependent atherosclerosis. Part of these effects may come through regulation of lipid and cholesterol metabolism, including the ability of SIRT1 to modulate the activity of the nuclear receptor LXR, a critical factor in reverse cholesterol transport. In addition, a conditional deletion of SIRT1 in endothelial cells has been demonstrated to impair the angiogenic response following an ischaemic insult.

Age is also associated with progressive decline in physical activity levels in a wide range of species, ranging from the *Caenorhabditis elegans* worm (87) to humans (88), with major metabolic consequences (89).

Therefore optimal therapeutic intervention to antagonize aging should prevent cell death and accumulation of senescent myocytes, eventually leading to decrease in occurrence of adult heart diseases (90).

The well-documented beneficial effects of exercise occur in a paradoxical background of biochemical framework, represented by increased production of potentially harmful substances such as reactive oxygen and nitrogen species, other free radicals, acids and aldehydes (91 - 95). Prolonged exercise then leads to the formation and accumulation of acids and other metabolites, which are potentially powerful damaging agents (4).

The most available data on SIRT1 and exercise have been obtained by studying skeletal muscle tissue. As skeletal muscle is a major site of glucose utilization, correlation data support the hypothesis that insulin resistance is related to age-associated reductions in muscle mitochondrial function (96, 97). Several studies have reported that increased physical activity can improve mean life span presumably by reducing mortality risk from many age-related diseases, including cardiovascular disease, stroke, type 2 diabetes, and certain cancers (98 - 103). In contrast, exercise and longevity studies in rodents (99, 100) and humans (104) have failed to document an exercise effect on maximum life span. For example, it was found in rats that exercise improved survival compared with sedentary ad libitum-fed controls, but did not result in life span extension (43, 99, 100, 105). Furthermore, when exercising rats were matched for body weight with food-restricted paired weight sedentary rats, only the food-restricted rats had an increase in maximum life span (99, 106).

However, it has been demonstrated that aerobic exercise is able to partially reverse the typical age-associated decline in muscle function, in particular by increasing skeletal muscle mitochondrial oxidative capacity (107), in particular through an increase in citrate synthase activity and shift of the myosin heavy chain fiber type to a more oxidative phenotype (108).

In adult humans, an aerobic exercise program improved insulin sensitivity, mitochondrial enzyme activity, and mixed muscle protein synthesis (109). Despite similar enhancement of muscle mitochondrial function in response to aerobic exercise training, younger people increased insulin sensitivity more than older people, indicating dissociation between increases in insulin sensitivity and mitochondrial function. Aerobic exercise has been reported to enhance muscle mitochondrial biogenesis through a calcium-regulated signaling pathway (110). Chronic aerobic exercise has also been shown to stimulate 5-AMP-activated protein kinase (AMPK) activity with subsequent increases in fatty acid oxidation and glucose uptake in skeletal muscle (111, 112). There is also evidence that chronic chemical activation of AMPK increases mitochondrial enzyme activity in selected skeletal muscle, suggesting a possible role of AMPK in mitochondrial biogenesis (113). Despite the fact that such adaptations have been analyzed for several decades, the exact mechanism behind the effects of exercise on increasing mitochondrial function remains incompletely defined. Chen *et al.* showed that Sirt1 is required for such an increase during physical activity. In particular, a parameter of mammalian calorie restriction, up-regulation of physical activity, requires the gene that codes for Sirt1. The molecular mechanism for this increase in physical activity is not known. It is possible that calorie restriction triggers changes in brain regions that govern physical activity and that Sirt1 is a regulator of this pathway (114).

SIRT1 plays a role in muscle gene expression in the modulation of the cytosolic NAD+-to-NADH (reduced form of nicotinamide adenine dinucleotide) ratio (115). Because the cytosolic NAD+-to-NADH ratio changes during muscle contraction (116), it is possible that SIRT1 contributes to skeletal muscle adaptations with endurance exercise. Recently Koltai *et al.* (117) found that exercise training increased the activity, but not the level of SIRT1 in skeletal muscle of both young and old rats. In particular, exercise training reverses the decreases in Nicotinamide phosphoribosyltransferase (NAMPT) and NAD content that occur during aging, and this is thought to be mediated by increased SIRT1 activity. The authors concluded that regular exercise training attenuates the aging process of skeletal muscle via the NAMPT-NAD+-SIRT1 pathway.

Suwa *et al* showed that both the SIRT1 and PGC-1α protein expression was higher in the red, slow-twitch, and oxidative muscles than in the white, fast-twitch and glycolytic muscles and correlated highly with the mitochondrial components, such as cytochrome C (118). It is therefore possible that both SIRT1 and PGC-1α play a role in maintaining the muscle fiber type–specific manner of mitochondrial oxidative capacity. The deacetylation activity of SIRT1 in the skeletal muscle is considered to increase during exercise (119), thus suggesting that exercise promotes the deacetylation of PGC-1α. Collectively, it is possible that skeletal muscle metabolic adaptations with endurance training

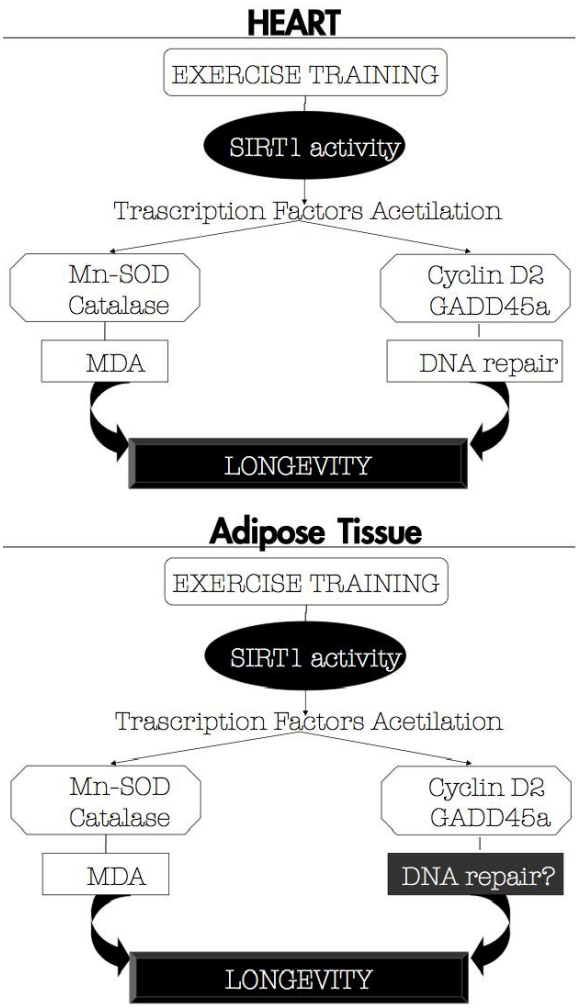

Figure 1. Scheme of possible mechanism involving exercise training in increasing lifespan. In left ventricle exercise training enhanced FOXO3a protein expression. This was associated with a decrease in cyclin D2 and an increase in GADD45a mRNAs in the heart of aged rats. In adipose tissue an increase in FOXO3a protein expression and a decrease in cyclin D2 but no changes in GADD45a mRNAs was found, probably in relationship to higher oxidative stress in this tissue that would induce the adipocytes to choose apoptosis or necrosis rather than repair as mechanism of detoxification. Therefore like caloric restriction, exercise training increases SIRT1 activity, which in turn could be responsible for the beneficial effects of training on age-related systems impairment. In particular the hypothesis is that exercise training could promote oxidative stress response via FOXO3a action.

are associated with the posttranslational regulation of PGC-1α independently of the PGC-1α protein content. In addition, SIRT1 also interacts with several other proteins (120), and it regulates forkhead transcription factors (FOXOs) transcriptional activity (22, 121), which would contribute to the expression of genes involved in fatty acid oxidation (122). The SIRT1 also interacts with p53 (120), which regulates mitochondrial respiration (123). These observations raise the possibility that SIRT1 interacts and deacetylates other proteins, as well as PGC-1α, regulating metabolic adaptations (see section "Role of sirtuins in caloric restriction"). In addition, a previous study demonstrated that increased expression of SIRT1 improved the insulin sensitivity in skeletal muscle by repressing protein-tyrosine phosphatase 1B expression (124), a well-known negative regulator of the insulin-signaling pathway (125). These results suggest that the effect of endurance exercise training for improving insulin sensitivity partially results from increased SIRT1 protein expression in skeletal muscle.

One potential candidate to explain the mechanisms concerning the exercise-induced increase of the SIRT1 protein is nitric oxide synthase (NOS). Nisoli *et al* demonstrated that energy restriction in wild-type mice increased both the SIRT1 expression and mitochondrial biogenesis in several tissues, whereas such changes were largely diminished or did not occur in endothelial NOS nullmutant mice (126), thus suggesting that energy restriction–induced SIRT1 expression and mitochondrial biogenesis could in large part depend on NOS. Both the NOS activity and neuronal NOS (nNOS) protein expression in skeletal muscle is induced by muscle contraction with electrical stimulation *in vivo* (127). Acute endurance exercise enhances the phosphorylation of nNOS in human skeletal muscle (128). Three to four weeks of endurance swimming training to rats increases the Ca2+- dependent NOS activity and the nNOS protein expression in the quadriceps femoris muscle (129). Collectively, these results raise the possibility that endurance exercise increases skeletal muscle NOS activity and nNOS expression and then they can induce the SIRT1 expression. Another candidate is AMPK. Endurance exercise increases AMPK activity in skeletal muscle (128, 130). A single administration of AMPK activator AICAR to rats significantly increases the SIRT1 protein in the extensor digitorum longus muscle (Suwa M, Nakano H, and Kumagai S, unpublished data).

Moreover, Ferrara *et al.* (131) demonstrated that a prolonged moderate exercise training is able to reduce oxidative stress levels and to induce an increase in SIRT1 activity in the heart and adipose tissue of aged rats, suggesting that chronic exercise, by inducing SIRT1 activity, exerts an antioxidant effect. Because it is known that exercise training exerts its beneficial effects particularly on the cardiovascular the authors also tested FOXO3a and its targets involvement in the heart of aged trained rats, showing that exercise training enhanced FOXO3a protein expression. This was associated with a decrease in cyclin D2 and an increase in GADD45a mRNAs in the heart of aged rats. In adipose tissue an increase in FOXO3a protein expression and a decrease in cyclin D2 but no changes in GADD45a mRNAs was found, probably in relationship to higher oxidative stress in this tissue that would induce the adipocytes to choose apoptosis or necrosis rather than repair as mechanism of detoxification. Therefore we suggested that, like caloric restriction, exercise training increases SIRT1 activity, which in turn could be responsible for the beneficial effects of training on age-related dysfunctions. In particular we hypothesize that exercise training could promote oxidative stress response via FOXO3a action (Figure 1).

7. CONCLUSIONS

Exercise training could represent an useful tool, not only for prevention but also for therapy of aging related diseases. In fact, prolonged exercise training is a recognized tool for rehabilitation of cardiovascular patients, in particular after myocardial infarction, by improving cardiovascular functions and physical work capacity (132), in prevention and treatment of several conditions related to endothelial dysfunction, by decelerating the aging processes of the arterial wall and of the endothelial functions (133). In particular, the effectiveness of prolonged and moderate exercise training could be explained by hormesis, defined as the life supporting beneficial effects resulting from the cellular response to single or multiple rounds of mild stress (4, 134-137).

Therefore, a prolonged and moderate training program could be useful also to elucidate the underlying molecular mechanisms of aging, and the SIRT1 pathway could be considered the tool by which the exercise training works to counteract the age-related oxidative stress damage.

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