Controversial Impact of Sirtuins in Chronic Non-Transmissible Diseases and Rehabilitation Medicine

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Running title: Epigenetics of physical exercise

Abstract: A large body of evidence report about the positive effects of physical activity in some pathophysiological conditions associated with age. Physical exercise alone or in combination with other medical therapies, unquestionably, causes reduction of symptoms in chronic non-transmissible diseases often leading to significant amelioration or complete healing. The molecular basis of this exciting therapeutic outcome, however, remain obscure. Epigenetics, exploring at the interface between the environment and the remodelling of chromatin structure, promises to shed light on this intriguing matter possibly contributing to the identification of novel therapeutic targets. In this review, we shall focalize on the role of class III histone deacetylases (HDACs), sirtuins (Sirt), which altered function has been frequently associated to the pathogenesis of aging-associated diseases including cancer, cardiovascular, muscular, neurodegenerative, bones and respiratory diseases, often with a controversial role. Numerous studies, in fact, demonstrate that Sirt-dependent pathways are activated upon physical and cognitive exercises linking the modulation of mitochondrial function, DNA structure and gene expression to designed medical therapies eventually leading to tangible beneficial outcomes. However, in similar conditions, other studies assign to sirtuins a negative pathophysiological role. Nevertheless, to study sirtuins in chronic diseases might lead to improve life quality in the elderly.

Keywords: epigenetics, rehabilitation, DNA methylation, histone modification, HDAC, exercise, health span, heart failure, neurodegeneration, cancer, lung fibrosis, bone formation.
1. **Sirtuins and their pleiotropic properties: an overview**

Sirtuins (Sirts) are a family of evolutionarily conserved class III histone deacetylases originally discovered in *S. Cerevisiae*, named Silent Information Regulator 2 (Sir2) and conserved to humans [1]. Biochemically, they act predominantly as ADP-ribosyl-transferases requiring NAD⁺ and an acetylated lysine substrate to produce a molecule of nicotinamide and O-acetyl-ADP ribose, which in turn can be used as a metabolic messenger by the cell [2, 3]. The mammalian Sir2 family is composed by seven members (Sirt1-7) that differ from each other for specificity, catalytic activity and cellular localization depending on cell, tissue type, metabolism and stress conditions. All Sirts share a conserved domain called Sirtuin Core Domain, formed by 250-260 amino acids, which retain catalytic activity and NAD⁺-binding site. Sirts differ at their N and C terminus domains that confer differences in molecular weight and perhaps substrate specificity. These HDACs are involved in multiple functions controlling chromatin structure and integrity [4], inhibition of senescence and apoptosis [5], metabolic homeostasis [6], cell differentiation and development. Despite all of these activities, Sirts’ main role seems that of cellular redox sensors. In response to stress signals, in fact, they get activated and most of them deacetylase lysine residues on histones, especially H4K16Ac and H3K9Ac [7], but they can also remove acetyl groups on non-histone proteins such as p53 [8] and tubulin [9]. More in deep, Sirt1, the best characterized Sirt member, is the only one with a clear role in genomic silencing through heterochromatin formation. The Sirt1 main biochemical form is that of a homo-trimer which is localized predominantly into the nucleus [10]. Here, Sirt1 deacetylases not only H4K16Ac and H3K9Ac but also H1K26Ac [10], the di-trimethyltransferase Suv39h1 [11], FOXO [12], NfkB [13], p53 [14], Ku70 [15], eNOS [16], PGC-1α [17], MyoD, PCAF [18], UCP2 [19] and PPARγ [20] to name a few. As a result, cell survival is enhanced as well as DNA repair. In some physiopathological conditions, such as cardiac hypertrophy, and during tumorigenesis Sirt1 activates the protective signaling pathways controlled by AKT and PDK1 [21]. In HeLa and HEK293T cells it has been shown that the main function assigned to another Sirt member, Sirt2, is the deacetylation of cytosolic α-tubulin via HDAC6 interaction [9] which causes the inhibition of active intracellular transport [22]. A temporary nuclear Sirt2 localization was found during G2/M transition marked by H4K16Ac deacetylation [7]. Also, in mouse erythrocytes, it has been demonstrated that Sirt2-dependent deacetylation activates glucose six phosphate dehydrogenase (G6PDH) which stimulates the pentose phosphate pathway to supply cytosolic NADPH under oxidative stress [23]. In response to the insulin signal, it has been shown that Sirt2 is activated by AMPK stimulating its interaction with Akt which in turn undergoes to deacetylation and activation [24]. Remarkably, this Sirt has been found involved in apoptosis regulation. When Sirt2 is down-regulated, in fact, the level of chaperonin 14-3-3 ζ is increased and sequester the pro-apoptotic protein BAD in the cytoplasm, preventing its localization into mitochondria a necessary step towards apoptosis [25]. In vivo, using mouse models, Sirt2 has been found to deacetylate and stabilize BubR1, a mitotic checkpoint kinase which leads to an increase in lifespan [26].

Mitochondria are another relevant cellular compartment in which Sirts play important regulatory roles. Sirt3 deacetylates some mitochondrial proteins involved in crucial metabolic pathways [27]. For example, under stress conditions, Sirt3 migrates from the nucleus to mitochondria where, by deacetylation, the sirtuin increases the enzymatic activity of Acetyl-CoA Synthase 2. This enzyme becomes active and enhances the ratio of mitochondrial/nuclear protein localization in favor of mitochondria [27]. In normal growth conditions Sirt3 coexits in both nuclear and mitochondria compartments [28], but in the nucleus, Sirt3 is associated with Sirt2 predominantly contributing to H4K16Ac deacetylation and chromatin remodelling [28].

Sirt4 and Sirt5 are other mitochondrial specific sirtuins. In particular, Sirt5 acts as a protein modifier removing succinyl and malonyl groups from lysine [29, 30]. Similarly, Sirt4 does not have any deacetylating function, but it acts on mitochondrial proteins, such as glutamate dehydrogenase (GDH), as ADP-ribosylase. In consequence of Sirt4 function, GDH results inactivated [31].

Sirt6 and Sirt7 are localized into the nucleus where their activity is that of an ADP-ribosyltransferase. Specifically, Sirt7 is nucleolar and interacts with RNA Polymerase I for the transcription of ribosomal genes [32], activates the nuclear respiratory factor (NRF) isoform α/β complex via deacetylation of NRFβ1 regulating mitochondrial biogenesis and function [33]. It also activates the testicular nuclear receptor 4 (TR4) which transcribes genes involved in lipid metabolism increasing fatty acid uptake and triglyceride synthesis/storage [34]. Meanwhile, Sirt6 is involved in genomic integrity, it is a chromatin-associated
deacetylase and is ubiquitously expressed from early to adult stages [35]. In this sub-compartment, Sirt6 interacts with a putative tumor suppressor and negative regulator of cell proliferation known as a GCIP factor. As a result, Sirt6 stimulates DNA repair or proliferation arrest when genomic integrity is compromised [36].

2. Sirtuins and physical exercise in cancer.

The role of Sirt1 in cancer remains unclear probably in consequence of the disease heterogeneity. Some evidences, in fact, support a Sirt1 role as tumor suppressor while others suggest for its oncogenic potential. In colon cancer, Sirt1 suppresses cancer development via a feedback loop in which c-Myc is involved. c-Myc binds Sirt1 gene promoter increasing sirtuin expression, while Sirt1 deacetylates c-Myc that loses its DNA affinity [37]. This tumor suppressor activity has been confirmed in vivo after Sirt1 overexpression in a mouse model of colon cancer. In this setting, the animals showed a four-fold reduction in size and number of adenomas in the small intestine and colon [38]. On the contrary, in human colon cancer, Sirt1 is often overexpressed, especially in more advanced stages [39]. This contradiction might be perhaps explained by Sirt1 deacetylase activity on p53 which determines a weak DNA-p53 interaction that causes loss of DNA repair capacity [14]. Furthermore, Sirt1 may negatively control the expression of p16/INK4A while promoting Rb phosphorylation, cell proliferation and reduction in cellular senescence as seen in human embryonic lung fibroblasts [40]. Recently, it has been demonstrated that Sirt1 has a pathogenic role in breast cancer in which it is upregulated promoting cell proliferation. In this contest, Sirt1 modulates the p-AKT/AKT ratio in favour of the phosphorylated form of the molecule which becomes active promoting cellular division. Again in breast cancer Sirt7 expression has been found elevated in early disease stages compared to advanced cancer [53]. In addition, prostate, bladder carcinoma, glioblastoma, and ovarian cancers with mutated BRCA1, all show a reduction of Sirt1 expression compared to normal tissues [41]. The biological consequences of this down-regulation are still unclear. Interestingly, positive evidence about the effect of physical activity on people affected by colon cancer has been described. A study reports that the beneficial effects of exercise are dose-dependent [42]. Aerobic exercise up to 300 minutes per week significantly decreased tumor biomarkers such as the prognostic vascular adhesion molecule 1 compared to patients whose followed a low dose exercise program [42]. Whether this positive effect on cancer patients might depend on sirtuin it is still controversial, however, the activation of sirtuins that occurs upon exercise might play an important role in this condition. In fact, in spite of the apparent pro-oncogenic properties, Sirt1 seems also important for an efficient DNA-break repair. Cells and mice knocked down for Sirt1, exhibited DNA damage–induced aneuploidy with an efficiency of DNA break repair reduced by approximately 50% [41]. Although at the moment it is very speculative, based on the evidence, the genetic targeting by siRNA or other approaches of Sirt1, Sirt7 or other Sirts in combination with physical exercise might represent an innovative therapeutic approach potentially beneficial in cancer. This is an important question worth of further investigations. In line with this consideration, in a recent meta-analysis, Sirt3 emerged as a negative prognostic biomarker for tumors such as chronic lymphocytic leukemia, breast cancer, colon cancer, hepatocellular carcinoma and renal carcinoma [43]. For Sirt4, 5, 6, and 7, at present, there are only incomplete evidence about whether they may be relevant in cancer or not [44].


Of the 56.4 million deaths worldwide in 2015, 54% were due to cardiovascular diseases (CVD) [45]. In this context, it has been clearly shown that physical activity and a healthy lifestyle significantly decrease the risk of CVDs and their worsening [46]. Accordingly, a large number of cardiac rehabilitation (CR) programs has been developed to treat CVDs attempting to prevent secondary events. In CVDs, CR consists of at least three phases [47]. The first, is performed during the acute phase, and is aimed at preventing/reducing immediate complications. In a second phase, operators stabilize patient’s symptoms and conditions. Finally, patients enter in a long term CR and follow-up program [47]. Nowadays, the most important CVD that may interest old people is chronic heart failure (CHF) and a body of literature demonstrated the beneficial effects
of physical activity in this category of patients [48]. In fact, to take under control this severe pathophysiological condition, for which a cure does not exist, it is important to follow a detailed exercise program at least for two/five times per week, 45/60 minutes each session [48]. After this training an improvement of the quality of life and physical function has been consistently reported [48]. Notably, in untrained CHF patients, Sirt1 in significantly down-regulated, a phenomenon that has been seen also in cardiac hypertrophy in the presence of pressure overload [49]. In a recent study, the beneficial effect of exercise in CHF patients has been investigated throughout and at the end of an intense rehabilitation program by collecting blood samples to evaluate Sirt1, superoxide dismutase (SOD) and antioxidant catalase (Cat), activity in peripheral blood mono-nucleated cells and in plasma samples respectively [50]. The result of this trial showed an increase of Sirt1 and Cat compared to control levels, while SOD was reduced [50]. Additionally, this study evaluated the effect of patients’ serum on the senescence process of endothelial cells (EC). Specifically, ECs were challenged with serum of HF patient taken at the end of rehabilitation training. In this condition, a strong reduction of senescence biomarkers was observed compared to controls [50]. To better understand the role of Sirt1 in this process, Sirt1 was inhibited determining an increase of EC senescence and a decrease in Cat activity [50]. These data clearly contribute the evidence that Sirt1 is activated during CR in CHF patients, however, further experiments on a larger cohort of subjects should be necessary to better understand the contribution of Sirt1 in CHF.

Biologically, Sirt1 results pivotal during heart development, especially during the second heart field formation in which Sirt1 silences ISL1 driving the differentiation of cardiac progenitor cells into cardiomyocytes [51]. Afterwards, Sirt1 level declines by 80% since the beginning of organogenesis, remaining at that level during adulthood [52]. In contrast, Sirt1 is highly expressed in endothelial cells in which it upregulates endothelial nitric oxide synthase (eNOS) expression and function, contrasting the development of atherosclerosis, smooth muscle cells senescence, inflammation and accumulation of ROS in arteries thus supporting vascular growth [16]. Once activated, Sirt1 reduces cholesterol biosynthesis in hepatocytes and macrophages [53] and upregulates the liver X receptor (LXR) [54]. The deacetylation mediated by Sirt1 on LXR, in fact, results in a decrease of plasma HDL levels due to the enhancement of reverse cholesterol transport and balancing of fatty acids metabolism leading to a global lipid reduction in serum [54]. Moreover, it has been shown that a functioning Sirt1 decreases inflammatory cytokines, TNFα, intercellular adhesion molecule (ICAM)-1, interleukin (IL)-6, IL-1, and inducible NOS (iNOS) expression in macrophages and endothelial cells [55, 56]. In this context, when Sirt1 deacetylates FOXO (a negative regulator of blood vessels) this transcription factor became inactive and the generation of new blood vessels is facilitated [57]. Other evidence shows that Sirt1 is downregulated during ischemia/reperfusion injury (I/R) [58]. In experiments performed on cardiac specific Sirt1-deficient mice, the animals exhibited heart damage similar to that caused in humans by I/R [59].

Notwithstanding the unquestionable evidence of a positive impact of Sirt activation on heart development and in the amelioration of CVDs worsening, Sirt1 upregulation has been also associated with many pathophysiological conditions such as those determined by nutrient starvation, pressure overload, and ischemic preconditioning. In pressure overload, nuclear Sirt1 activates PPARα which binds to the oestrogen-related response element, resulting in the repression of genes controlling mitochondrial function and cardiac contraction [49]. In cardiac hypertrophy, Sirt1 may act in the cytosol, where it deacetylates Akt and PDK1, PDK1 phosphorylates Akt which becomes active and promotes the hypertrophy [21]. Consistently, in Sirt1-deficient mice it has been shown that after physical exercise cardiac hypertrophy was reduced [21]. Further, in heart failure it has been demonstrated that Sirt1-PPARα complex is able to downregulate oestrogen related receptor response element targets promoting mitochondrial dysfunction [49]. In conclusion, similarly to cancer, Sirt1 might have protective or deleterious effects on the heart apparently depending on the pathophysiological environment or specific stressor triggers. Again additional experience it is necessary to understand in which condition Sirts are beneficial and when, instead, they should be silenced to achieve positive therapeutical effects.

To modulate Sirts' activity, some natural molecules are available. The most important activator known so far is Resveratrol (Res), a polyphenol founded in grapes, blueberries, raspberries, and mulberries, which seems to have a protective effect on the heart [60]. Through direct and indirect mechanisms, Res stimulates Sirt1 to deacetylase targets such as PGC-1α [61] resulting in regulation of fatty acid storage and glucose metabolism. Also, Res can activate AMPK which directly phosphorylates eNOS causing an increase of nitric
oxide (NO) production, vessel dilatation [62] and reduction of ROS levels. Simultaneously, Res-activated Sirt1 physically interacts with eNOS and deacetylates lysines in the calmodulin-binding domain of eNOS, leading to enhanced eNOS activity [63]. Recently, it has been demonstrated the Res activity on renin-angiotensin system in which the polyphenol decreases the activity of prorenin receptor (PRR)-angiotensin converting enzyme (ACE)-Ang II axis in the aorta. The action of Res, enhancing ACE2 -Ang-(1 and 7)-Ang II type 2 receptor (AT2R)- Mas receptor (MasR) axis determines a cardiovascular positive effect [64]. The combination of Res and exercise has been found beneficial on vascular function reducing inflammatory markers and enhancing vessel responsiveness [65]. However, a recent work reported that the beneficial effect of resveratrol could be due to an antioxidant effect without any evidence of Sirt1 activation [66].

For reasons still undetermined Sirt2 has been found important maintaining cardiac homeostasis counteracting hypertrophy and failure. In fact, during pathological hypertrophy, Sirt2 levels are significantly reduced in the human heart, and this condition has been confirmed by Sirt2 deficient animals in which agonist-induced hypertrophy is exacerbated while Sirt2 overexpression attenuates hypertrophy in cardiomyocytes [67]. In case of Sirt2 reduction, cardiac remodeling apparently occurs in consequence of missing interaction between Sirt2, that cannot migrate into the nucleus in G2/M phase, and the transcription factor NFAT isoform C2 (NFATc2) which is not deacetylated resulting in increased transcription activity of foetal cardiac genes which contribute to hypertrophy [67]. In vitro, the downregulation of Sirt2 in embryonic myoblasts enhances anoxia-reoxygenation stress tolerance sequestering Bad and contrasting the progression of cell death [25]. In HeLa and HEK-293T cells it has been reported that Akt binds directly Sirt2 instead of PI3K activating Akt pathway during glucose deprivation [24]. Moreover, the effects of Sirt2 on Akt activation, like Sirt1, suggest that its activation could prevent cardiac hypertrophy [24]. However, as for other aforementioned pathophysiological conditions, in adult tissues the protective role of Sirt2 is not well characterized yet.

In consequence of its important mitochondrial function, Sirt3 is linked to congenital heart diseases characterized associated with mitochondria dysfunction. It has been shown, in fact, that in Friedreich's ataxia (FRDA), an early onset (at 10-15 years of age) autosomal recessive disease characterized by low levels of NAD+, there is not enough Sirt3 activity. The normalization of the NAD+/NADH ratio in FRDA increases Sirt3 function contributing to the amelioration of cardiac function and suggesting that Sirt3 can be a therapeutic target in pediatric FRDA patients [68]. In the mouse model of Sirt3 deficiency, the evidence suggests that the increase of mitochondrial permeability transition pore (mPTP) [69] and TGFβ1 signalling [70] are associated with cardiac hypertrophy and interstitial fibrosis detectable at early life stages.

In the presence of heart hypertrophy, Sirt3 results overexpressed causing an increase of MAPK/ERK, PI3K/Akt and Ras pathway activity as well as an intracellular increase in ROS levels. In consequence, genes involved in cell growth are activated while antioxidant genes, such as manganese superoxide dismutase (MnSOD) and Cat, become repressed [71]. In contrast, a protective effect of Sirt3 on cardiomyocytes apoptosis has been observed upon overexpression. Sirt3, in fact, may act on Ku70 which sequesters Bax, a pro-apoptotic protein, from mitochondria [72]. Furthermore, Sirt3 contrast doxorubicin-induced cardiomyopathy by activating O-guanine-DNA glycosylase-1 (OGG1), an important mitochondrial DNA repair enzyme [73]. Again, it appears that the protective or detrimental effect of Sirt3, as for other members of this important molecular family, might depend on the pathophysiological context.

Little is known about Sirt4 and Sirt5 in CVDs. However, their protective effects on neonatal cardiomyocytes have been recently reported. Specifically, Sirt4 overexpression in H9C2 cells is leading to an increase of procaspase/caspase ratio and a decrease of Bax translocation to mitochondrial membrane [74]. Sirt5 knockdown, instead, decreases the viability of neonatal cardiomyocytes, enhancing the activity of caspase 3 and 7. During oxidative stress, Sirt5 binds Bcl-xl blocking the apoptotic cascade and increasing cell survival [75] thus suggesting the regulation of its expression as a novel treatment of the oxidative stress-related cardiac injury. Sirt6 has been found to be downregulated in failing human hearts. Sirt6 is a key regulator of cardiac hypertrophy and heart failure controlled by IGF-Akt signalling through c-Jun and the deacetylation of Histone 3 at lysine 9 [76]. In the context, it has been shown that Sirt6 overexpression protects against cardiac hypertrophy by the physical interaction with c-Jun which becomes inactivated and the transcription of genes involved in IGF signalling is inhibited [76]. On the other hand, in Sirt6 deficiency, the hyper-acetylation of H3K9 increases c-Jun activity and cardiac hypertrophy worsening heart failure [76]. In hypoxia, the overexpression of Sirt6 activates AMPK pathway, increases Bcl2 protein level, inhibits NFκB,
decreases ROS formation and p-Akt activity [77]. In this condition, cardiomyocytes are protected from hypoxic stress and apoptosis [77]. Moreover, in the presence of oxidative stress, Sirt6 deacetylases and ADP-ribosylates PARP1 and activates the non-homologous end joining and homologous recombination repairing of DNA double-strand breaks (DSB) [78]. About Sirt7, it has a cardioprotective role influencing Akt and p53 pathways in cardiomyocytes [79]. It has been shown that reduction of Sirt7 expression and activity leads to Akt and p53 hyper-acetylation in mice causing cardiac hypertrophy and cardiomyopathy accompanied by extensive fibrosis associated to heart failure [79]. At the moment, the picture is still too fragmented and little or no information is available about the effect of physical exercise on sirtuins in general and specifically on Sirt4, 5, 6, 7. More research in this direction it is necessary to clarify this important aspect.

4. The involvement of sirtuins in other pathophysiological conditions relevant to rehabilitation medicine: The Chronic Obstructive Pulmonary Disease (COPD)

The chronic obstructive pulmonary disease is a progressive lung disease that includes emphysema, chronic bronchitis, refractory (non-reversible) asthma, and some forms of bronchiectasis. It is characterized by progressive and largely irreversible airflow limitation, which is associated to an abnormal inflammatory response in the lung. The major risk factor to COPD is cigarette smoke (CS) that increases the inflammatory state in lungs even in smokers without COPD [80]. Other risk factors are from the environment (dust, air pollution, and second-hand smoke) or associated with genetic conditions such as those determining the alpha-1 deficiency-related emphysema [81]. Currently, COPD is treated to reduce dyspnoea by increasing physical stress tolerance using bronchodilator drugs [82]. Indeed, COPD patients can be admitted to a pulmonary rehabilitation program in which they follow a specific physical activity at least five times a week made of postural and aerobic exercises, breathing and respiratory muscle training and upper- and lower-body muscle strength exercises [83]. At the end of such a training program of about four weeks, some parameters are measured to compare with the original values [83]. During this training, dyspnoea, exercise capacity, walking distance and pulmonary capacity significantly improves and all patients report about a general amelioration of clinical symptoms [83]. Of interest for this review, a recent study demonstrates a significant correlation between COPD rehabilitation program and epigenetic modifications [84]. After 24 training sessions, in fact it was found that, HDACs activity increased [84] as well as increased all anti-inflammatory signals associated with TGFβ reduction and upregulation of IL-6 [84].

A progressive worsening of inflammation is typical of COPD patients, and Sirt1 seems to play a positive role in this context negatively regulating NFkB through deacetylation and inactivation. Sirt1 expression, in fact, decreases under the effect of cigarette smoke, facilitating the release of pro-inflammatory cytokine [85]. In lungs of rats, it has been demonstrated that cigarette smoke could activate the transcription of inflammatory genes through chromatin remodeling [86]. However, in humans the most relevant cell types involved in COPD pathogenesis are macrophages and neutrophils, both secreting chemokines and matrix metalloproteinases [87]. To elucidate the role of Sirt1 in macrophages infiltrating the lung of COPD patients, peripheral lung samples were collected and compared with non-smoker and smoker without COPD [88]. Sirt1 level and activity significantly decreased in smokers, and COPD patients and this evidence has been associated with the post-translational modification of the Sirt1 including the introduction of 4-hydroxy-2-nominal (4-HNE) and tyrosine nitration. In parallel, a significant increase of NFkB subunits (RelA/p65) activity has been observed in macrophages and endothelial cells often associated to the release of significant amounts of IL-8 [88]. Whether Sirt1 plays a role in this context remains to be determined. However, it must be noted, that different results have been obtained studying cells coming from large and small airways such as macrophages, lymphocytes and endothelial cells [89]. In a study, three groups of patients were compared: COPD smoker, smokers without COPD and healthy no-smokers. It has been observed that in the large airways of COPD patients and smokers, Sirt1 expression was lower than in healthy people. On the contrary, in the small airways of COPD patients, a significant reduction of Sirt1 levels was found compared to the lung of smokers and healthy controls [89]. In addition, the exposure to chronic cigarette smoke, in COPD patients, enhanced matrix metalloproteinases 2, 9 and 12 and this phenomenon was associated with low Sirt1 level too [90]. Interestingly, MMP9 activity is under control of tissue inhibitor of MMP (TIMPs) which N-terminal lysine acetylation is dependent on Sirt1 [91]. Hence, as for other chronic non-transmissible diseases, in COPD Sirt1 and/or TIMPs expression could be considered as potential novel pharmacological targets [90].
Endothelial progenitor cells (EPCs) have been studied in COPD patients’ blood. Also here, downregulation of some genes such as Sirt1 mRNAs, CD31, CD34, and miR-126-3p emerged clearly, while the overexpression of miR-34a was observed only in comparison with signals from healthy smokers [92]. Remarkably, Sirt1 protein and its fragments has been found in COPD serum [93]. Specifically, Sirt1 120KDa (S120) has been found fragmented in COPD patients compared with healthy smokers or non-smokers subjects [93]. All of these observations suggest the possibility to use CD31, CD34, Sirt1 mRNAs, miR-126-3p, miR-34a [92] and S120 [93] as biomarkers for a better detailed diagnosis of COPD.

In human bronchial epithelial cells, the expression of an antioxidant enzyme, the heme-oxygenase-1 (HO-1), is normally low [94]. However, CS extract increases HO-1 level through NFE2-related factor 2 (Nrf2) activation [94]. Remarkably, HO-1 level is controlled by Sirt1. Consistently, it has been shown that the neutrophil elastase does not block Nrf2, but it can cleave Sirt1 in lung epithelial cells resulting in a CS-induced downregulation of HO-1 [94].

Frequently, in COPD patients, cognitive impairment is observed. A study explored the use of Di-3n-Butylphthalalide (NBP), a plant extract known for its therapeutic effects on cognitive effects in a COPD cognitive impairment rat models [95]. As a result, the Sirt1/PGC-1α pathway was activated and slowed the progression of cognitive degeneration in animals [95]. In spite of its generally positive effect in COPD, it must be said that a Sirt1 polymorphism, in the Muğla population, has been recently associated with COPD exacerbation. Specifically, the AG genotype of rs7895833 and CC rs2273773 has been found more frequently recurrent in COPD patients than GG AG and TT TC respectively [96]. Hence, as said before for other conditions, also in COPD the role of Sirt1 remains controversial and additional studies are necessary to achieve a better understanding about the general involvement of Sirt1 in the disease pathogenesis and their potential role in novel therapeutic strategies.

About other sirtuins, Sirt4 was down-regulated in human pulmonary microvascular endothelial cells treated with CS extract, meanwhile the expression of E-selectin, and that of the vascular cell adhesion molecule 1 (VCAM1) significantly increased [97]. Remarkably, the attachment of monocytes to lung endothelium promotes the development of a pro-inflammatory environment [98]. In this context, the overexpression of Sirt4 seems sufficient to prevent the inflammatory reaction. Indeed, Sirt4 inhibits the degradation of IκBα, an inhibitor of NfkB [97] preventing the activation of the inflammatory cascade and acting along its pathway may represent a novel therapeutic approach to COPD progression. Whether Sirt4 could be considered as a promising target for COPD treatments awaits more investigations to be ascertained.

5. Sirtuins in neurodegenerative disorders of interest for rehabilitation medicine.

Neurodegenerative diseases are a wide range of conditions in which neuronal cells progressively lose their normal activity and die. This phenomenon alters mental function and often limits motility causing a progressive and incurable debilitation. These diseases are of interest for rehabilitation medicine although the molecular mechanisms underlying the positive effect of physical and cognitive training are still unresolved. The most important mental disorder is dementia which affects a significant number of older adults over the age of 85. Alzheimer’s disease represents 60-70% of all cases of dementia [99] whereas other relevant neurodegenerative pathologies are Parkinson’s disease, motor neuron diseases, prion disease, huntington’s disease, spinocerebellar ataxia and spinal muscular atrophy, to mention some of the most important. Briefly, Alzheimer’s disease (AD) is characterized by deposition of brain amyloid plaques and neurofibrillary tangles which rise in consequence of amyloid precursor protein (APP), presenilin 1 (PS1), presenilin 2 (PS2), tau protein and apolipoprotein E (APO E) gene mutations or altered processing. These proteins accumulate into extracellular matrix forming deposits which change the matrix homeostasis and interfere with physiological impulse transmission [99]. In this disorder, it has been suggested that Sirt1 is probably the most important sirtuin involved in neuroprotection which prevents or reduces accumulation of β amyloid. Here, neuronal Sirt1 overexpression or neuronal Sirt1 resveratrol-dependent activation decreased a Ser/Thr Rho kinase, which is involved in amyloid β metabolism [100]. Additional studies demonstrated that the deacetylating effect of Sirt1 on Tau’s lysine made this molecule accessible to ubiquitin ligases driving Tau to degradation and significantly slowing the disease progression [101]. Further studies demonstrated the direct effect of Sirt1 on PI3K/Akt pathway determining Tau hyper-phosphorylation in a human neuroblastoma cell line. Here, Sirt1 downregulation by siRNA approach significantly decreased cell survival in consequence of
inhibition of Akt phosphorylation. In this work, it was found that Sirt1 localizes close to the plasma membrane modulating Akt activity and decreasing β amyloid accumulation upon Tau hyperphosphorylation [102].

Along an independent line of research, a recent study demonstrated the effect of Sirt3 on p53. As well known, in physiological condition deacetylated p53 prevents DNA damage and mitochondrial dysfunctions, but Alzheimer’s patients exhibit a decrease of Sirt3 which is associated to an accumulation of acetylated p53 in the mitochondria of frontal cortex neurons. In this context, the direct deacetylation of mitochondrial p53 on lysine 320 by Sirt3 had neuroprotective effect on Alzheimer’s disease slowing its progression [103].

Interestingly new approaches to reduce AD symptoms include physical and cognitive exercises. In a recent clinical trial, the feasibility of a music-supported video-based exercise was evaluated in AD patients [104]. Exercises were progressively increased in a four weeks program, and physical parameters such as warming up exercise, strength, static and dynamic balance, functional exercises, endurance, and flexibility were recorded [104]. Here, it was observed an amelioration of exercise quality at the end of the program and patients were satisfied and enjoyed the training [104]. Whether sirtuins activation, which occurs upon physical exercise as often discussed in this review, played a positive role in this context remains to be ascertained.

In developed countries, it is common to find Parkinson’s disease (PD) in the population over 60 years of age. Typical signs of this pathology are tremors of the head, hands and limbs, rigidity, bradykinesia and postural instability gathered to cognitive and psychiatric disorders [105] which cannot be cured. However, surgery and drugs can reduce some symptoms and recently it was found that transcranial alternating current stimulation decreased motor symptoms [106]. As known, Parkinson’s disorder is consequence of altered dopamine secretion caused by cell death in the substantia nigra and to the formation of Lewy’s bodies (intracellular α synuclein accumulation) secreted by living neurons [107]. Also in this condition, Sirt1 seems acting as a neuroprotector through the activation of the heat shock factor 1 (HSF1) which enhances transcription of molecular chaperones such as heat shock protein 70 [108]. In PD, the effect of Res on Sirt1 and AMPK determines an increase in mitophagy in dopaminergic neuronal cells. In fact, Res stimulates the clearance of injured mitochondria and enhances the degradation of transgene α synuclein through autophagy inhibiting the formation of Lewy’s bodies [109]. In addition, in a PD mouse model, it was demonstrated the regulation of Sirt1 by Cdk5 through ubiquitin-proteasome pathway in which Cdk5 seems more expressed than in normal controls causing the loss of neuronal reactivity [110]. Interestingly, a small molecule called AGK2, which was identified as a potent Sirt2 inhibitor, showed a neuroprotective effect rescuing neurons from toxicity consequent to α synuclein accumulation [111]. The same effect was obtained in vivo in a Sirt2 deficient mouse model [112]. In light of this contradictory results it remains unclear whether sirtuins play a positive or negative role in PD and further studies are necessary to elucidate this point.

Recent in vivo and in vitro studies indicate that microRNAs regulate Sirt3. Specifically, miR-494-3p, which is enriched in PD neurons, binds Sirt3-3’UTR determining Sirt3 downregulation which is associated with motor neuron impairment in a PD mouse model. The discovery of this Sirt3/miR-494-3p circuitry can be of interest to find a treatment for PD by using miR-494-3p as a target [113].

A large body of evidence suggest for a significant improvement of PD symptoms after physical activity. One of the most recent trials introduced “Ai Chi” exercises, a Japanese aquatic therapy, to the rehabilitation program for mild and moderate PD patients [114]. After five weeks of training, an improvement of mobility, balance, motor ability and quality of life was observed compared to the group which followed a land-based program [114]. This study suggests that this discipline, instead of the traditional exercises, might have a positive effect reducing PD symptoms. Whether molecular mechanisms involving sirtuins activation are involved in this effect remains to be clarified. However, the PD mouse model suggested for an involvement of Sirt1 as neuro-protector agent after aerobics or some physical exercise [115]. In this case, it was shown that the mitochondrial complex I activity and Sirt1, both typically decreased in the hippocampus of PD mice, were rescued upon exercise improving the general physical conditions of the animal [115]. Moreover, in trained PD mice, lower levels of pro-inflammatory cytokines were observed suggesting for an action of Sirt1 on NF-κB pathway which resulted inhibited [115]. Taken all together, these findings positively indicate the that physical exercise might have an epigenetic effect increasing sirtuins activity in the presence of concomitant PD symptoms leading to their amelioration.

Nowadays, the most common type of motor neuron disease is the amyotrophic lateral sclerosis (ALS),
ALS is caused by a slow and progressive neurodegenerative process of neurons in the brain and in spinal cord resulting in loss of coordination, speech, eating and even breathing. The most common ALS form is the sporadic one which affects 90-95% of ALS cases [116] although a less common inherited form has been reported [117]. The real pathogenesis is still not well understood, but it seems linked with wide number of mutations in various genes such as superoxide dismutase [118], TAR DNA-binding protein 43 [119], FUS RNA binding protein [120] and C9orf72 [121] to name the most commonly reported. To better understand the role of sirtuins in ALS, different experiments were performed in which Sirt1 was stimulated using resveratrol. In some experiments, rat neuronal cells were incubated with ALS patient cerebrospinal fluid (CSF) or with CSF from a healthy donor. As a result, rat neuronal cells were more damaged than control, but whenever resveratrol had been added, the toxic effects of CSF from ALS patients was reduced [122]. As in Parkinson’s disease, in ALS it was demonstrated that the activation of Sirt1 deacetylates the heat shock factor 1 (HSF1) upregulating hsp70 and hsp25 thus prolonging the lifespan of motor neuron [123]. Another sirtuin, the mitochondrial Sirt3, was shown to be neuroprotective in ALS studies. SOD1 mutation leads to ALS phenotype causing mitochondrial dysfunction and fragmentation. A transgenic mouse model of SOD1-mutation was used to test Sirt3 efficiency [124]. In the mouse motor neurons, Sirt3 overexpression protected against mutant SOD1 harmful effects preventing mitochondrial damage and neuronal cell death [124].

A number of clinical trials showed the benefit of physical exercise in ALS patients. In a recent study, a significant increase of the score of the functional independence scale was observed in ALS patients after a specific program training in which improvement of oxygen consumption, muscle strength and fatigue was reported [125]. An ALS-like condition was observed in old people [126] where physical exercise determined re-innervation of muscle fibers at neuromuscular junctions normally reduced by aging [126]. Whether these beneficial effects might be consequence of sirtuin activation require further investigations.

As it will emerge from what is discussed below, in Huntington’s disease (HD) the role of sirtuins seems the most controversial. HD is an autosomal dominant illness characterized by loss in coordination and motility, variable personality and psychiatric disturbances combined with cognitive dysfunction. The patients’ genotype is characterized by CAG repeats expansion that encode glutamine residues in Huntington protein which aggregates into the cytoplasm [127]. This illness is highly disabling, and first symptoms arise commonly in middle-aged adults (35-44 years) [128]. Some HD features are similar to other neurodegenerative diseases of old people such as the accumulation of aggregates, cognitive decline and behavior changing [128]. A significant neuroprotection effect was obtained from Sirt1 overexpression in C. elegans after polyglutamine cytotoxicity [129] However, in Drosophila Sirt1 and Sirt2 downregulations were associated to neuron survival suppressing the disease [130]. In contrast, in a transgenic mouse model overexpressing a truncated N-terminal Huntington protein, the deletion of Sirt1 catalytic site worsened Huntington’s disease symptoms while the overexpression of Sirt1 reduced protein aggregation improving physiological conditions [131].

Mechanistically, it has been proposed an effect of Sirt1 on TORC1 deacetylation which normally interacts with CREB. As a result, in the Huntington mouse model, TORC1-CREB complex is interfered by mutated huntingtin protein, and the brain-derived neurotrophic factor transcription that supports neuronal growth, survival, and differentiation cannot be transcribed [131]. The upregulation of Sirt1 rescues this defect enhancing the formation of TORC1-CREB complex [131]. Additionally, Sirt2 was investigated in Huntington mouse model, but its downregulation does not have any effect on disorder’s condition and does not block Huntington protein intracellular stacking [132]. However, Sirt3 activation upon viniferin stimuli was found to increase AMPK function in the mouse model of Huntington’s disease and in consequence the neurodegeneration was attenuated [133].

In an attempt to find a therapy that can improve HD symptoms, the effect of β-Lapachone (βL), a naturally derived compound present in the Tabebuia avellanedae roots, known to be beneficial as anti-bacterial, antiviral, anti-cancer, anti-inflammatory and beneficial for wound healing too, was tested in the HD mouse model [134]. The HD animals were injected with βL, and its effect on Sirt1, CREB, and PGC-1α was investigated. It was found that Sirt1 and p-CREB increased, while PGC-1α acetylation was reduced in the brain of treated mice. Altogether, treated animals showed an improvement of the rota-rod score compared to HD controls and an amelioration of Huntington protein deposit with consequent reduction of HD phenotype [135].

Recently, the introduction of a rehabilitation program has been suggested for HD patients. A clinical trial
positively evaluated the effect of physical activity on progression of speed, endurance walking, exercising balance, muscular strength, pulmonary and cognitive functions [136]. It is currently unknown whether these interventions activated the sirtuin pathway. However, because HD is a genetic illness, it must be expected that Sirt1 stimulation and/or exercises, could only be considered as a palliative intervention awaiting for the genetic defect correction.

6. Conclusions.

Epigenetics is a new field constantly under investigation in which DNA structure is involved in gene regulation without altering the primary gene sequence. During human development, DNA undergoes numerous structural modifications such as cytosine methylation or chromatin remodeling by acetylation/deacetylation and methylation/demethylation of histone tail lysine residues. Physiologically, all of these changes drive cells to differentiation or introduce modifications of their functional state. However, in a large number of pathophysiological conditions, the alteration of chromatin structure described so far ends up with changes in the normal pattern of gene expression. In pathophysiology, to restore the physiological state, a body of evidence supports that a disease-specific physical activity program may help modifying our epigenetic landscape, improving function of cardiovascular, muscle, respiratory, gastrointestinal, nervous and immune systems. Recent studies demonstrated the role of sirtuins, the class III HDAC enzyme family, in different diseases in which they are downregulated or are not working properly. Although the role of sirtuins remains controversial, at least in specific pathophysiological conditions including cancer or neurodegenerative diseases. It is remarkable how numerous observations reported that sirtuins’ level and activity would increase upon intense exercise training contributing to ameliorate physical and cognitive parameters such as walk resistance, memory, and muscular strength. With this review, necessarily limited to those aspects of the role of sirtuins more easily amenable to chronic non-transmissible diseases of interest for rehabilitation medicine, we attempted to raise attention toward the regulation of these NAD+-dependent deacetylases combined with physical training. In conclusion, more attention should be paid to sirtuins that should be investigated with more attention in the context of physical rehabilitation training programs of illnesses associated to aging.
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