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Oxidative stress, mitochondrial dysfunction, and respiratory chain enzyme defects in inflammatory myopathies

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ABSTRACT

We investigated the relationship between oxidative stress and inflammatory myopathies. We searched in the current literature the role of mitochondria and respiratory chain defects as sources of oxidative stress and reactive oxygen species production that led to muscle weakness and fatigue. Different molecules and pathways contribute to redox milieu, reactive oxygen species generation, accumulation of misfolded and carbonylated proteins that lose their ability to fulfil cellular activities. Small peptides and physical techniques proved, in mice models, to reduce oxidative stress. We focused on inclusion body myositis, as a major expression of myopathy related to oxidative stress, where mitochondrial abnormalities are causative agents as well. We described the effect of physical exercise in inclusion body myositis that showed to increase strength and to reduce beta amyloid accumulation with subsequent improvement of the mitochondrial functions. We illustrated the influence of epigenetic control on the immune system by non-coding genetic material in the interaction between oxidative stress and inflammatory myopathies.

1. General aspect of oxidative stress in inflammatory idiopathic myopathies (IIM)

1.1. Mitochondrial dysfunction and respiratory chain enzyme defect in inflammatory idiopathic myopathies

It is well established that mitochondria represent the cell's powerhouse, due to their central role in bioenergetics, metabolism, respiratory cell activity, and calcium homeostasis especially in highly energetic and dependent tissues (including muscle, central nervous system, lymphoid organs, or glands). A deregulation of their functions has been speculated as a crucial molecular hallmark underlying different conditions, among them inflammatory idiopathic myopathies (IIM). IIMs are a spectrum of rare and heterogeneous autoimmune disorders dominated by proximal muscle weakness. Mitochondrial abnormalities can trigger, by their own, from bioenergetic failure and oxidative stress production to protein accumulation, inflammation, and cell death, features that are present in this range of disorders [1].

At this regard, through the years, several cellular pathophysiological mechanisms have been proposed to explain Idiopathic Inflammatory Myopathies (IIM) (Table 1).

In 1995, Campos et al. [2] first highlighted defects of respiratory chain enzyme complexes in patients with IIM trough the analysis of muscle biopsies of 4 women with IIM. Three of them had both histochemical evidence of mitochondrial proliferation and combined defects of the respiratory chain complexes, indicative of mitochondrial

Abbreviations: IIM, Idiopathic Inflammatory Myositis; IBM, Inclusion Body Myositis; ROS, Reactive Oxygen Species; NO, Nitric Oxide; ETC, Electron Transport Chain.

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Table 1
Main characteristics of principal studies on mitochondrial dysfunction in myositis.

Reference	Patients	Controls	Main results
Camposet al., [2] 1995 Spain	Four consecutive women with IIM	no	Three patients had mitochondrial proliferation in non-regenerating muscle fibers; these patients had defects of respiratory chain enzyme complexes
Arenaset al., [3] 1996 Spain	13 consecutive patients with IIM (7F and 6 M)	25 age-matched healthy controls	High levels of free carnitine and carnitine esters in the muscles of patients with IIM and abnormal carnitine distribution was detected in 11/ 13 patients
Chariotet al., [4] 1996 France	30 patients with IIM (15 with DM, 12 with PM, and 3 with IBM)	30 age-matched controls	Focal COX deficiency was detected in 24 of 30 patients (13/ 15 with DM, 8/ 12 with PM, and 3/ 3 with IBM) and in 18/ 30 controls. Correlation between capillary loss and COX deficiency
Blumeet al., [5] 1997 USA	Ten patients with histologically typical PM except for an excess of muscle fibers with absent COX staining	Twelve patients (10 with DM and 2 vacuolar myopathies) with normal numbers of muscle fibers and normal COX activity	The presence of an excess of COX-negative muscle fibers in myositis patients correlated with the presence of mtDNA deletions in muscle. The mtDNA deletions occurred in 90% of patients with PM and COX-negative muscle fibers
Ceaet al., [6] 2002 UK	Nine patients with DM and 5 with PM	18 age-matched healthy individuals	Post-exercise 31P-MRS indices of muscle oxidative metabolism were all impaired in DM and PM. In both groups of patients, the phosphocreatine and adenosine diphosphate recovery half-times were almost twice as long as in controls and the maximum rate of mitochondrial ATP production was half as compared with normal subjects
Alhatouet al., [7] 2004 USA	Twelve patients with DM	Twelve control patients with neurogenic atrophy	Eleven of 12 patients with DM showed histochemical evidence of mitochondrial dysfunction in perifascicular distribution
Meyeret al., [8] 2017 France	15 untreated DM patients	Fourteen patients with non-DM inflammatory myopathies and 16 sedentary controls	The transcript levels of 1537 genes were increased in DM patients, whereas those of 956 genes were decreased. The main up-regulated gene cluster encoded proteins involved in inflammatory responses. The main cluster of down-regulated genes encoded proteins involved in mitochondrial integrity and function
Basualto-Alarcón C et al., [10] 2020 Chile	Five patients clinically diagnosed with IIM	Four patients who underwent shoulder surgery	Oxygen consumption rate increased in IIM derived cells reaching values similar as those observed in control cells, showing a preserved metabolic flexibility However, the ability of IIM cells to adapt to this metabolic stress did not result in increased cellular fitness and endurance to stress
Zhaoet al., [11] 2022 China	86 PM/DM patients	110 controls	The mtDNA of PM/ DM patients showed D-loop (displacement loop) of SNP (Single nucleotide polymorphisms) accumulation. The distribution frequencies of 16304C (associated with lower IL-4 levels) and 16519C (higher IL-2 expression) were significantly higher in the patients with PM/DM. Reactive oxygen species (ROS) generation was increased in PM/DM patients
Hedberg-Oldforset al., [12] 2022 Sweden	Twelve patients with DM (5 juvenile DM; 7 adult DM)	14 individuals, investigated for a possible muscle disorder, but in whom the clinical and pathological investigations excluded a muscle disease	Deficiency of complexes I and IV in the perifascicular regions with enzyme histochemical COX deficiency, whereas succinate dehydrogenase activity and complex II were preserved in muscle biopsy specimens of IIM patients

Abbreviations: 31P-MRS: 31P-magnetic resonance spectroscopy; ATPase: Adenosine 5'-TriPhosphatase; DM: Dermatomyositis; ETC: Electron transport chain; IIM: Idiopathic Inflammatory Myositis; mt-DNA: mitochondrial DNA; PM: Polymyositis; SNP: Single nucleotide polymorphisms.

dysfunction. Patients with IIM may show mitochondrial proliferation in muscle biopsy specimens, defined by the presence of ragged red fibers or subsarcolemmal accumulation of oxidative elements. Biochemical analysis of muscle respiratory chain enzymes in patients with IIM had never been carried out before.

Additional evidence for mitochondrial dysfunction comes from studies on muscle concentrations of carnitine. L-Carnitine therapy is claimed to be effective in patients with primary mitochondrial diseases, but the value of this therapy in patients with inflammatory myopathy and carnitine deficiency remains to be proved. Carnitine has a key role in regulating the transport of long chain fatty acids into mitochondria and modulates intracellular concentrations of free coenzyme A. In plasma and tissues, carnitine is present in free form and as acylcarnitine esters. The same group, coordinated by Arenas et al. [3], had analyzed the levels of free carnitine and carnitine esters in the muscles of patients with IIM and abnormal carnitine distribution was detected in 11 of 13 patients. Impaired mitochondrial function could produce acylCoA accumulation and increase esterification of carnitine, leading to a low level of free carnitine. Other mechanisms besides mitochondrial dysfunction could account for the carnitine insufficiency. Defective beta-oxidation or increased glycolytic activity could result in an excess of acylCoA and acetylCoA groups, respectively. Also, decreased uptake of active carnitine from plasma by muscle could reduce intracellular carnitine levels. In this scenario, therapy with L-carnitine may be useful [3].

In the same years, Chariot et al. [4] has shown through histological studies of skeletal muscle biopsy from 30 patients (15 with dermatomyositis, 12 with polymyositis, and 3 with inclusion body myositis) and 30 age-matched controls the presence of ragged-red fibers, cytochrome *c* oxidase (COX)-negative fibers and increased succinic dehydrogenase staining. Histoenzymatic reaction for cytochrome *c* oxidase (COX), the complex IV of mitochondrial respiratory chain, can be a more sensitive marker than ragged-red fibers formation to assess mitochondrial abnormalities, and is used with increasing frequency in the diagnosis of acquired mitochondrial disorders. In some patients with inclusion body myositis and polymyositis, COX reaction showed partial deficiency, associated or not with mitochondrial (mt) DNA deletions. These changes are more pronounced and consistently reported in s-IBM (sporadic Inclusion Body Myositis) than in DM and PM. In DM patients, abnormal numbers of ragged red fibers and COX-negative fibers were shown to be more frequent than in PM patients. They demonstrated a correlation between capillary loss and COX deficiency and suggested that mitochondrial dysfunction in dermatomyositis could be due to ischemia. The mitochondrial dysfunction and COX deficiency can occur in inflammatory myopathies. Such a mitochondrial dysfunction is not solely related to the aging process. They suggested that muscle ischemia could contribute to mitochondrial dysfunction in dermatomyositis [4].

The study of Blume et al. [5] suggests that the activity of COX in muscle fibers should be studied whenever polymyositis is suspected on clinical or pathological grounds. They found that the presence of an excess of COX-negative muscle fibers in myositis patients is correlated with the presence of mtDNA deletions in muscle. The mtDNA deletions occurred in 90% of patients with polymyositis and COX-negative muscle fibers. They found that patients with polymyositis and an excess of COX-negative muscle fibers, but no inclusion bodies, have common features including selective quadriceps weakness, mitochondrial pathology (through histochemical and DNA analysis) and a poor response to immunosuppressive therapy. COX staining of muscle may provide a rationale for discontinuing immunosuppressive treatment when revaluating patients with otherwise typical PM who are unresponsive to therapy. The mitochondrial abnormalities could either be a primary event with subsequent myopathy and inflammatory infiltration, or secondary to the inflammation, muscle fiber degeneration and regeneration [5].

Several studies have tried to understand if mitochondrial dysfunction is the cause or the consequence (due to inflammation and reduction of the flow of oxygen) of IIM. A quantitative 31P-magnetic resonance spectroscopy and MRI study by Cea et al. [6] suggested that in DM and PM the oxidative metabolism deficit (mitochondrial dysfunction) is secondary to impaired blood supply.

Mitochondria are very sensitive to hypoxia and react early to hypoxic stress. In fact, Chariot et al. [4] found that the pattern of capillary loss correlated positively with the distribution of COX-deficient myofibers in patients with dermatomyositis. Alhatou et al. [7] confirmed that the abnormalities of succinate dehydrogenase (SDH) and COX staining indicate abnormalities of mitochondrial function in the atrophic perifascicular fibers, these mitochondrial changes are likely early events in tissue ischemia.

Meyer et al. [8] showed that mitochondrial functional defects are a hallmark of DM. In their study, a transcriptomic analysis of early untreated DM muscles revealed that the main cluster of down-regulated genes was mitochondria-related. Histochemical, electron microscopy, and in situ oxygraphy analyses showed mitochondrial abnormalities, including increased ROS production, and decreased respiratory activity, which was correlated with low exercise capacities and a type I IFN signature. Moreover, IFN-β induced ROS production in human myotubes was found to contribute to mitochondrial disfunctions. Thus, these data highlight the central role of mitochondria and ROS in DM. Mitochondrial dysfunctions, mediated by IFN-β induced-ROS, contribute to poor exercise capacity. In addition, mitochondrial dysfunctions increase ROS production that drive type I IFN-inducible gene expression and muscle inflammation and may thus self-sustain the disease [8]. The review by Paik et al. [9] confirmed the upregulation of both type I interferon (IFN)- and type II INF-inducible genes as key features of both adult DM and juvenile DM (JDM). In myocytes of patients with DM, robust expression of both type I IFN- and type II IFN-inducible genes correlates with expression of genes associated with inflammation and regeneration. The various approved and investigational JAK inhibitors have distinct pharmacologic activity at four human JAK isoforms (JAK1, 2 and 3, and Tyrosine kinase 2). Several are known to strongly inhibit JAK1 and/or TYK2 and accordingly inhibit types I and II IFN signaling. The patients displayed persistent refractory symptoms and did not see any improvement with several first- and second-line treatments (e.g., methotrexate, mycophenolate, azathioprine), including corticosteroids and other immunomodulatory agents such as intravenous immunoglobulin. The treatment with a JAK inhibitor was associated with a wide range of significantly improved or resolved DM manifestations, including skin lesions, muscle weakness, interstitial lung disease, and calcinosis. Most patients with DM were able to taper or discontinue concomitant corticosteroid therapy while on JAK inhibitor therapy, further supporting the therapeutic potential of JAK inhibitors in DM [9].

In contrast to this study, Basualto-Alarcón et al. [10] asserted that in human derived IIM myoblasts, mitochondria are not dysfunctional and still retain their ability to adapt and respond to environmental signals, at the cost of letting cells more prone to death after a specific, oxidative, insult. Interestingly, IIM-derived cells showed a tendency to die in higher proportions than controls because of this metabolic stress. This "mitochondrial flexibility" seemed to suggest that this organelle is still capable of detecting and transducing environmental signals, to maintain the relation between mitochondrial function and cellular signaling. Nonetheless, the fact that higher death rates were observed for IIM cells lighted on the role that mitochondria might play in the weakness and atrophy in patients with IIM [10].

Mitochondrial functional defects are a hallmark of IIM. Zhao et al. [11] found that increased mtDNA copy number may induce mitochondrial dysfunction, thereby initiating the onset of PD/DM, and SNPs (Single nucleotide polymorphisms) accumulated excessively in patients with PM/DM. The mtDNA of PM/DM patients showed D-loop (displacement loop) of SNP accumulation. The D-loop region correlates with the entire mtDNA replication and transcription; therefore, genetic alterations in this region may affect mitochondrial function through excessive ROS generation and alteration of the immune status [11].

Mitochondrial dysfunction plays an important role in muscle weakness and fatigue in DM. Indeed, patients show a reduced aerobic capacity and there is histopathological and biochemical evidence of oxidative phosphorylation dysfunction mainly affecting perifascicular regions. Hedberg-Oldfors et al. [12] found that the respiratory chain complexes I and IV, which contain mtDNA encoded subunits, were deficient in perifascicular regions, whereas the entirely nuclear encoded complex II was unaltered in parallel with an unchanged mitochondrial density. The respiratory chain dysfunction in DM muscle is associated with mtDNA depletion causing deficiency of complexes I and IV, which are partially encoded by mtDNA, whereas complex II, which is entirely encoded by nuclear DNA, is preserved. The depletion of mtDNA indicates a perturbed replication of mtDNA explaining the muscle pathology and the disturbed aerobic metabolism [12].

1.2. The involvement of nuclear factor-kappa B (NF-kB)

Histologically IIM are characterized by inflammatory cell infiltrations and upregulation of major histocompatibility complex I (MHC-I) within muscle cells and upon their surfaces [13]. Increased oxidative stress and activation of nuclear factor-kappa B (NF-kB) is involved in the pathogenesis of autoimmune diseases such as systemic lupus erythematosus and IIM [14]. NF-kB has multifaceted role, and it is involved in multiple processes (ranging from genes expression, cytokines and chemokines production, enzyme regulation etc.). In 2003 Monici et al. [15] were among the first to demonstrate NF-kB activity in DM and PM using immunocytochemistry, immunoblotting of nuclear extracts, and electrophoretic mobility shift assay (EMSA). Activation of the receptor for advanced glycation end products (RAGE) by its ligand N6-carboxymethyllysine (CML), the major advanced glycation end product (AGE) in human tissues and a marker for cumulative oxidative stress, promotes the activation of NF-KB which stimulates the production of proinflammatory cytokines and sustains the damage. Haslbeck et al. [16] described the colocalization of CML-modified proteins, RAGE and activated NF-KB in mononuclear cells and in regenerating muscle fibers both in polymyositis (PM) and dermatomyositis (DM). CML-modified proteins are generated by oxidative modification of proteins of necrotic muscle fibers. This may be due to an increased delivery of free radicals from inflammatory cells during muscle fiber destruction or by post-necrotic liberation of peroxisomal enzymes like peroxidase or catalase. Authors hypothesized that the binding between CML and RAGE stimulated the intracellular oxygen radical production, necessary for NF-kB activation, and caused increased CML formation perpetuating the inflammatory processes [16]. In a small study of 64 DM patients, Yang et al. [17] analyzed the levels of lysyl oxidase-like 2 (LOXL2), an enzyme that catalyzes the oxidative deamination of lysines and hydroxylysines. They reported that its levels are upregulated in DM by NF-KB and through the PI3K/AKT/mTOR/HIF-1α pathway it promotes fibroblast differentiation and collagen deposition. Nevertheless, LOXL2 serum levels in DM patient with interstitial lung disease (ILD) were like DM-non-ILD patients (Fig. 1). Interestingly, in DM patients, LOXL2 was positively associated with disease activity assessed by Myositis Disease Activity Assessment Tool (MDAAT) [17].

1.3. Oxidative and antioxidant balance: bilirubin and uric acid

In support of the importance of oxidative stress and antioxidant status in the pathogenic mechanism of PM/DM, Chen et al. [18] measured serum concentration of bilirubin and uric acid in PM/DM patients comparing to healthy controls. Bilirubin and uric acid can reduce global oxidative stress reflecting the antioxidant status. Authors found reduced levels in PM/DM. Nevertheless, it was uncertain whether a low antioxidant status of bilirubin and uric acid were a cause or a consequence of the scavenging of elevated levels of reactive oxygen species [18].

1.4. Endoplasmic reticulum (ER) stress pathways and generation of reactive oxygen species (ROS)

The severity of muscle weakness does not strictly correlate with the degree of inflammatory cell infiltration because other non-inflammatory factors may be involved. Among them, the chronic activation of stress pathways of the endoplasmic reticulum (ER), also known as sarcoplasmic reticulum (SR), seem to play a major role [13]. Specimens from murine myositis model and from patients with myositis revealed the presence of ER-stress related markers such as Grp78, Grp75, Grp94 and Caspase 12 [19]. The ER-stress pathways promote the alteration of redox homeostasis and generate reactive oxygen species (ROS) which can cause oxidative damage to DNA, lipids, and proteins. Indeed, in case of ER stress, the accumulation of misfolded proteins promotes the

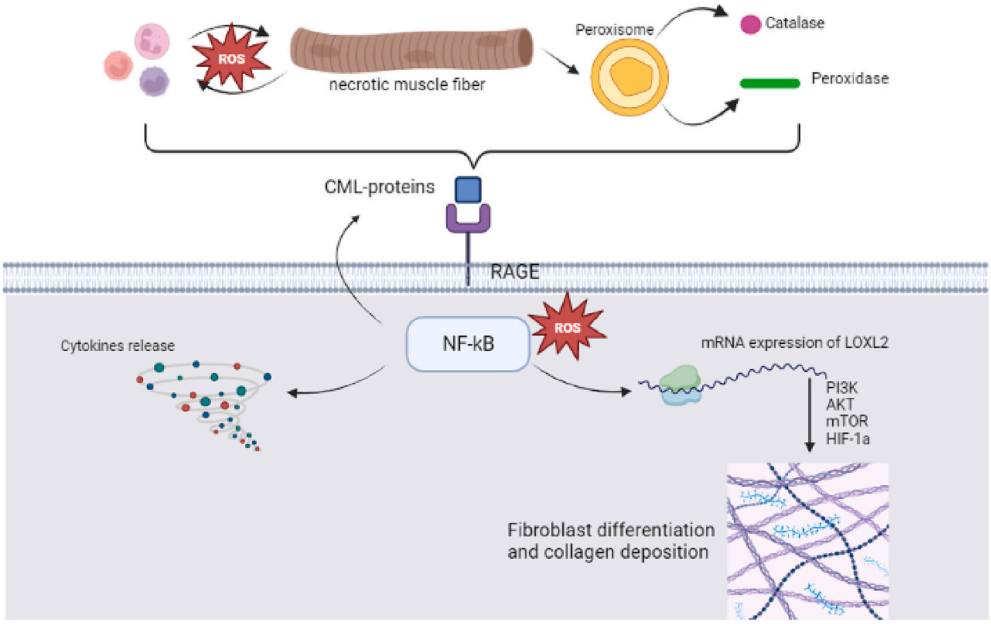

Fig. 1. The involvement of nuclear factor-kappa b (NF-kB).

production of H_2O_2 that crosses membranes, goes into cellular compartments, and carries out its oxidizing activity. Moreover, there is a tight crosstalk between ER/SR and mitochondria supporting ROS production [13]. However, ROS are also involved in cellular signaling, homeostasis and in mediating adaptive responses [20]. In case of cellular stress, ROS activity is perturbed with a consequent mitochondrial dysfunction, depressed force generation and activation of muscle catabolic and autophagy pathways [13]. Since chronic activation of ER stress pathways is described in IIM, and modified ROS generation is associated with muscle disfunctions other than IIM, Lightfoot et al. [13] investigated whether alteration of redox homeostasis, consequent to ER stress, may promote muscle weakness in IIM. They distinguished an acute phase during which H_2O_2, released from ER, reacts with free radicals and forms reactive species causing an oxidative damage to cytosolic components including contractile proteins. The chronic activation of ER stress responses perpetrates oxidative damage to the contractile structures impairing the force generation. Moreover, chronically elevated ROS generation may alter the electron transport chain (ETC) decreasing the rate of respiration, downregulating the ATP production with a consequent energy deficit [13].

1.5. Damage to proteins

With respect to protein damage, free radicals produced during oxidative stress can modify polypeptide chains leading to loss of protein function, as well as to the conversion of protein forms that are more susceptible to degradation by proteinases [21]. Generally, the generation of carbonylated proteins, i.e., carrying carbonyl groups, represent an important hallmark of oxidative stress. The protein-bound carbonyls may be formed either via metalcatalyzed oxidation (side chains of amino acids Pro, Lys, Thr and Arg), direct oxidation (Trp), lipid-peroxidation (Cys, Lys, His) and by glycation/glycoxidation (Lys, Arg). However, other advanced oxidation proteins products (AOPPs) can be formed, often by chlorinated oxidants such as hypochloric acid [22]. Moreover, direct oxidation of proteins can also occur [23].

The stable isotope labeling with amino acids in cell culture (SILAC) is a technique that allows the quantification of altered proteins. The SILAC methodology has been extended to in murine models of myositis and it identified abnormalities in levels of proteins involved in ER stress response, oxidative phosphorylation, glycolysis, cytoskeleton, muscle contractile apparatus and ubiquitin proteasome pathway (UPP). Authors found down-regulation of structural proteins, up-regulation of heats-shock proteins (HSP), superoxide dismutase (SOD) and peroxiredoxin suggestive of perturbations in stress response and redox signaling pathways. Regarding the UPP, in myositis it has been described an increased ubiquitination of muscle proteins as well as an increase in carboxyl-terminal hydrolase isozyme L1 (UCHL-1) that could be a marker for disease progression. The overexpression of class I major histocompatibility complex (MHC-I) on skeletal muscle fibers causes an accumulation of unfolded proteins in ER with a consequent ER stress response and down-stream activation of the endoplasmic-reticulum-associated protein degradation (ERAD) and UPP, and finally impacting on calcium homeostasis and energy metabolism and causing muscle degeneration. Interestingly, bortezomib, an inhibitor of evolutionarily conserved 26S proteasome, proved to prevent UPP pathway, to decrease the protein synthesis and afterwards the protein load in ER. The use of bortezomib showed a decrease in muscle inflammation and an improvement of muscle function in mice models.

The anti-inflammatory effect of bortezomib may be due to the attenuation of TNFα that is a target gene in NF-κB pathway and to the block of lymphocytes' transmigration [24].

1.6. Nitric oxide: one molecule, multiple actions

Since reactive oxygen intermediate (ROI) and nitric oxide (NO) are involved in cell injury and are generated during inflammatory processes, their action on muscle cells and on infiltrating inflammatory cells in IIM have been investigated [25]. Authors analyzed the effect of oxidative stress on apoptosis/necrosis. However, in IIM apoptosis does not seem to be involved in muscle fibers' death probably due to the not complete activation of the apoptotic cascade or to the anti-apoptotic effect of NO, instead, necrosis, mediated by inflammatory cells, plays a pivotal role. The direction towards apoptosis or necrosis is influenced by the level of oxidative stress: low levels favor apoptosis; high levels induce necrosis [25,26]. Since necrosis is associated with oxidative stress, Van Dooren et al. [27] investigated the oxidative modifications of the major autoantigen in myositis, histidyl-tRNA synthetase (HisRS or Jo1). They noticed that an increased oxidation status unexpectedly promoted its aminoacylation activity. However, they did not identify in patients' sera autoantibodies specifically targeting HisRS epitopes generated by these modifications [27]. The nitric oxide (NO) production and the enhanced expression of oxidative enzymes such as nitric oxide synthase (NOS) and its isoforms may lead to a redox milieu and may impact on cell death mechanisms and pathways in IIM [27]. NOS exists in three isoforms: neuronal NOS (nNOS) a constitutive isoform localized to the brain and skeletal muscle; endothelial NOS (eNOS) which, in human muscle tissues, is mainly found in endothelial cells; and inducible NOS (iNOS) regulated by cytokines and it can be induced in several cell types [28]. Tews and Goebel [26] showed that, in IIM, iNOS was up regulated in all kinds of muscle fibers, probably intensified by the inflammatory background, whereas nNOS presented an increased expression on the sarcoplasm of damaged as well as atrophic muscle [26]. Moreover, a study conducted by De Paepe et al. [28] described higher levels of iNOS not only on sarcolemma of distinct muscle fibers in polymyositis (PM) and sporadic inclusion body myositis (sporadic IBM) but also in endomysial infiltrates of PM and sporadic IBM, whereas perimysial inflammatory cell in dermatomyositis (DM) were mostly negative. These results support the hypothesis of a rapid synthesis of iNOS induced by the microenvironment that sustains the inflammation [26]. Wanchu et al. [29] reported increased levels of NO, as indicated by its surrogates markers such as citrulline and serum nitrite, among patients with PM/DM.

In tissues NO can react with superoxide free radicals and producing peroxynitrite. The latter in turn can react with free or protein-bound tyrosine, synthetizing 3-nitrotyrosine (3NT) a stable end-product. 3NT may be regarded not only as footprint of NO generation but rather as a potential measure of exposure to oxidative stress. The results of Zámečník et al. [30] reported high 3NT immunoreaction both in the endothelium (site of ROS generation) and in its close surrounding in inflamed areas of IIM (PM and DM). However, the endothelial cells were also positive in cases of confirmed non-inflammatory myopathies with secondary lymphocytic infiltration. These finding disqualified 3NT as an aid to the differential diagnosis of IIM.

In any case, nitric oxide (NO) has a multifaced role from cytoprotective to cytotoxic, it acts in inflammatory process, it has a signaling activity, it stimulates satellite cells to regenerate damaged muscle in myositis, and it may inhibit oxygen consumption causing a decreased ATP generation. The latter may explain why some experimental studies proved that increased NO in skeletal muscles caused a depression of the contractile function and consequent muscle weakness in patients with IIM [25,26].

Olivé et al. [31] described the accumulation of semicarbazide-sensitive amine oxidase (SSAO) in selected fibers in inflammatory myopathies, in IBM as well as other myopathies. SSAO deaminates both aromatic and aliphatic primary amines acting as a scavenger, and it is expressed at very low levels in human skeletal muscles. Authors found an overexpression in inflammatory and necrotizing myopathies. As these conditions are dominated by enhanced deamination, they supposed an increased function of the enzyme with subsequent catalytic activity which may trigger the production of ROS and oxidative muscle damage. Alternatively, the overexpression of SSAO may be a response to muscle injury [31].

1.7. Oxidative stress and vascular damage

Oxidative stress is tightly related to vascular damage that characterizes DM. High-density lipoprotein (HDL) protects endothelium from damage due to oxidized phospholipids, which increase under oxidative condition [32]. Bae et al. [33] found in IIM patients a higher myeloperoxidase (MPO) function responsible of abnormal HDL antioxidant activity. MPO promotes the formation of oxidation products of arachidonic acid and linoleic acid that not only oxidate low-density lipoprotein but also accumulate in HDL impairing their functions [32,34]. It is well established that oxidative stress and altered HDL function led to cardiovascular disease (CVD) whose incidence is higher in IIM patients than in general population [35,36]. The results of Bae and colleagues [33] suggest a pathomechanism for propagation of accelerated CVD and microvascular damage seen in IIM (especially in DM). Interestingly, Barsotti et al. [37] proposed noninvasive instrumental examinations (i.e. measurement of carotid parameters, AGE accumulation in the skin by autofluorescence and body composition by bioelectrical impedance analysis) to estimate and define the cardiometabolic risk in IIM even in the absence of traditional CV risk factors. Recently, Bae et al. [38] examined the activity of the HDL-associated antioxidant enzyme PON1 that promotes the antioxidant and anti-inflammatory function of HDL. They described suppressed arylesterase and lactonase activity of PON1 in IIM patients that correlated with higher disease activity and presence of severe ILD. Furthermore, they performed a genotype analysis that revealed the QQ genotype of PON1 Q192R polymorphism (a major determinant of the enzyme activity) associated with more favorable IIM outcome [38].

1.8. Therapeutic approaches

In living organisms, the antioxidant activity is carried out by the combined action of enzymes: catalase, glutathione peroxidase, and SOD [21]. However, due to the role of oxidative stress in IIM, the use of "broad spectrum" antioxidant had received significant attention but pursuant the side effects this approach did not appear applicable [39]. However, researchers investigated the possibility to develop compounds targeting specific cellular sites of ROS. For example, in mice models of muscle disfunction, supplementation with a small peptide called Szeto-Schiller 31 (SS-31), which is in the mitochondrial intermembrane space and associates with cardiolipin, proved to sustain ATP production, to reduce mitochondrial ROS production and to lower oxidative damage [40]. MitoQ, a ubiquinone derivative targeted to mitochondria, has attenuated mitochondrial dysfunction and in rodent model of amyotrophic lateral sclerosis improved muscle strength [41,42]. Other strategies involved molecules with upstream targets (e.g., salubrinal). Indeed, preventing the activation of ER stress pathways could interfere with ROS production and so mitochondrial and contractile dysfunction [43]. In other murine models of myopathy, the action of low-level laser therapy (LLLT) has been investigated. The principles of LLLT are based on photoreceptors of the mitochondrial respiratory chain, which change the membrane potential. Its effect is consequent to the generation of small amounts of ROS and antioxidants, changing the cellular redox state, reducing oxidative stress, and leading to biological responses. In mice, LLLT is responsible for changes in inflammatory biomarkers and oxidative stress: it decreases the levels of fibrinogen, L-citrulline and SOD, and it promotes muscle recovery in association with satellite cell proliferation and angiogenesis [44,45]. Similar results were obtained in experimental model of myopathy treated with pulsed electromagnetic field (PEMF) therapy, also known as magnetotherapy. The therapeutic effect derived from the antioxidant role of the electromagnetic field of the applied pulsed field [21].

Since hydrogen displayed effects on inflammatory and oxidative stress-mediated diseases in rodents, Ito et al. performed and open label trial of drinking 1.0 l per day of hydrogen enriched water (HEW) for 12 weeks in 14 patients with different muscle diseases (PM/DM, progressive muscular dystrophy, mitochondrial myopathies). In patients with PM/DM they reported a decrease of serum matrix metalloproteinase-3 (MMP3) and of serum triglycerides. MMP3 enhances T-cell migration, adhesion, and cytotoxicity by degrading extracellular matrix proteins in DM. The improvement of MMP3 levels is expected to ameliorate the pathogenic inflammatory process. Moreover, hydrogen may have a threshold effect or a dose-response effect and 1.0 l or more per day of HEW is necessary to carry out beneficial effects. Indeed, authors conducted a randomized, double-blind, placebo-controlled, crossover trial of 0.5 l per day of HEW for 8 weeks without observing any statistically significant effect [46]. Finally, it has been speculated about the role of 3-n-Butylphthalide (NBP) in individuals with myopathies. Previous research pointed out the multiple neuroprotective mechanisms of NBP in brain damage, including reducing oxidative stress and lowering lipid peroxidation, preventing neuronal apoptosis, and inhibiting neutrophils infiltration. In experimental models of autoimmune myositis, NBP seemed to increase the activity of SOD and catalase, to protect muscle mitochondria and muscle cells from oxidative damage and, to reduce muscle cells apoptosis [47].

The role of physical exercise and endurance activity on oxidative stress in IIM is often underrate. PM/DM patients have an impaired muscle oxidative efficiency, proved by the abnormal blood lactate levels at rest and after regular intervals from the end of sub-maximal aerobic exercise. However, after 6 weeks of aerobic training, lactate levels were reduced as well as the fatigue symptoms [48]. Munters et al. [49] in their randomized controlled pilot study, investigated the changes in muscle tissue proteome resulting from 12 weeks of endurance exercise. They described an activation of the aerobic phenotype through the up regulation of proteins involved in the oxidative phosphorylation pathway in mitochondria leading to a gain of function and ATP production. Moreover, they found a decrease in some genes involved in ER stress, observed by both RNA expression profiling and protesomics [49]. Similarly, it has been observed a lower fatigue resistance in experimental autoimmune myositis mice than in controls. These changes related to decreased activities of citrate synthase and cytochrome *c* oxidase (expression of mitochondrial oxidative capacity) and up-regulation of ER stress proteins (Grp78, Grp94 and PKR-like ER kinase). High-intensity interval training (HIIT) ameliorated all these alterations. HIIT fostered the peroxisome proliferator-activated receptor g coactivator-1a (PGC-1a), that is regarded as an important regulator of mitochondrial biogenesis and function and inhibited the increased amount of ER stress proteins. On the whole, these findings suggest exercise as an important non-pharmacological approach for diminishing ER stress and improving mitochondrial function [50].

2. Oxidative stress and muscle aging in inclusion body myositis (IBM)

IBM is the most common acquired myopathy after the age of 50. It is characterized by progressive asymmetric weakness predominantly affecting quadriceps and/or finger flexors. Muscle biopsies shows the distinct presence of rimmed vacuoles, congophilic inclusions, and protein aggregation [51].

2.1. The role of oxidative stress

Oxidative stress has been firstly hypothesized by Askanas et al. [52] as an important contributory factor leading to the IBM-specific muscle fiber destruction. Using an adenovirus vector, they transferred β-amyloid precursor protein gene into cultured normal human muscle fibers, inducing several aspects of the IBM phenotype, including vacuolization, mitochondrial cytochrome *c* oxidase deficiency, increased production of superoxide dismutase-1 and superoxide dismutase-1 mRNA, structural mitochondrial abnormalities including respiratory chain dysfunctions. Particularly, in this IBM-like experimental model, increased superoxide dismutase-1 and superoxide dismutase-1 mRNA suggested an attempted

protective response to pathologically increased free radicals like superoxide (SO) [52].

Moreover, in muscle biopsy specimens from patients with sporadic and autosomal (recessive and dominant) IBM, neuronal and inducible forms of nitric oxide (NO) synthase were found to be abnormally accumulated in vacuolated muscle fibers, along with nytrotyrosine, a product of proteins tyrosine groups' nitration due to the toxic molecule peroxynitrite (derived from the combination of SO and NO) [53]. The Authors [54] then postulated that the accumulation of β-amyloid precursor protein (βAPP), β-amyloid protein, or both within muscle fibers was an early step in the pathogenic cascade common to all forms of sporadic and hereditary IBMs, causing cellular disturbance leading to an increased generation of free radicals, culminating in oxidative stress. Abnormal lipid peroxidation was also demonstrated in sporadic IBM, e.g., the accumulation of malondialdehyde in vacuolated fibers [54].

In other important studies it was observed that, in muscle biopsies from IBM patients, nuclear factor kB (NF-kB) and Redox factor-1 (Ref-1), responsible for further βAPP gene transcription, were overexpressed in vacuolated muscle fibers, probably upregulated in response to oxidative stress [55,56].

Since insulin-like growth factor I (IGF—I) showed in vitro a protective role against oxidative stress and amyloid β toxicity, Broccolini et al. [57] investigated the role of IGF-I in sporadic IBM. They found IGF-I hyperexpression in a considerable amount of non- regenerating fibers in muscle biopsies from seven sporadic IBM patients, suggesting a response against to oxidative stress and amyloid β accumulation.

In 2008, Terracciano et al. [58] focused on DJ-1, a protein suspected to act as antioxidant and mitochondrial-protective agent, reported to be increased and highly oxidized in sporadic Alzheimer's disease and Parkinson's disease. They demonstrated that DJ-1 mutations, preventing its expression, to be a cause of early-onset autosomal recessive Parkinson's disease. On these bases, the Authors studied DJ-1 in sporadic IBM: DJ-1 was found to be increased in muscle fibers and mitochondria, in a highly oxidized form [58].

2.2. The role of muscle aging

Askanas et al. [52] also discussed the role of muscle aging in the development of IBMs.

For sporadic IBM, they postulated that, within aged muscle fibers, the overexpression of age-related genes, encoding detrimental cellular factors, or the down-regulation of youthful genes, encoding beneficial cellular factors, caused a reduction in cellular defence mechanisms leading to muscle fiber cellular death, vacuolar degeneration and atrophy. Another proposed hypothesis was the activation of a dormant virus in aged cellular milieu (due to decreased cellular defence mechanisms), causing mitochondrial abnormalities, oxidative stress, protein accumulations and altered transcription. In accordance with this concept, the Authors demonstrated the presence of HTLV p19 antigen detected immunohistochemically in vacuolated muscle fibers of a patient affected by sporadic IBM [52]. For hereditary IBM, the Authors postulated that causative abnormal genes, existing since birth, became manifest in the milieu of the early-adult muscle fibers, leading to vacuolar degeneration. In this context, the less-aged cellular environment was for the Authors a factor that could explain the less-advanced pathologic change observed in hereditary IBM, in comparison with sporadic IBM [52].

In 2010, Morosetti et al. [59] investigated about age-related abnormalities of satellite cells possibly accounting for the reduced regenerative potential of sporadic IBM myoblast observed in cultured cells.

They demonstrated that proliferation rate and clonogenicity of sporadic IBM cultured myoblasts were significantly lower and their doubling time was longer than that of normal age-matched controls. Moreover, telomere shortening was detected in sporadic IBM cells, along with increased active beta-catenin, this latter aspect reflecting stimulation of the Wnt signaling pathway, known to be activated in aged muscle. Beta-catenin was mainly localized within myonuclei. These observations suggested an early exhaustion of sporadic IBM muscles proliferative capacity and a premature senescence. Importantly, after many rounds of muscle growth, only sporadic IBM myoblasts accumulated congophilic inclusions and immunoreactive amyloid beta-protein 1–40 deposits [59].

2.3. The role of mitochondrial abnormalities

In 1993, Oldfors et al. [60] investigated skeletal muscle specimens from three patients with IBM. In all cases muscle fibers showed low or absent cytochrome *c* oxidase (COX) activity. Moreover, deleted mitochondrial DNA (mtDNA) was demonstrated to accumulate in COX-deficient fibers, and ultrastructural investigation showed abnormal mitochondria. Deletions in mtDNA probably occurred in COX genes and were responsible for COX-deficiency.

Two years later, the same Authors investigated the different types of mtDNA deletions present in isolated single COX-deficient muscle fibers from twenty IBM patients, and the relation between COX deficiency and muscle fibers regeneration. The deletions appeared to vary between each patient, although one 5 kb deletion was observed in all patients, the so-called "common deletion", already observed in about one third of cases with Kearns-Sayre syndrome, a congenital mitochondrial disease. These results indicated that the mitochondria with mutated mtDNA in a COX-deficient muscle fiber segment originate from one or a few mitochondria with this mutation. The Authors hypothesized that clonal expansion of mtDNA with mutations could occur during regeneration after segmental muscle fiber necrosis, since a regenerated muscle fibers originates from a small number of satellite cells which very few mitochondria. This hypothesis was supported by the fact that COX-deficient fibers were more frequent among fibers with positive immunostaining for Leu-19, a regeneration marker [61].

As above mentioned, in 1996 Askanas et al. [52] transferred β-amyloid precursor protein (βAPP) gene into cultured normal human muscle fibers, inducing several aspects of the IBM phenotype. Particularly, mitochondrial cytochrome *c* oxidase (COX) deficiency was observed, along with structural mitochondrial abnormalities, including significant enlargement, disorganization, paucity of cristae, para-crystalline inclusion, vacuolization, and accumulation of amorphous material. The degree of abnormality correlated with the duration of β-amyloid precursor protein gene expression. However, mitochondrial COX deficiency and structural abnormalities were not secondary to the other recognized aspects of βAPP-induced muscle degeneration, because they were present earlier in muscle fiber lacking other signs of degeneration [52].

The mtDNA deletions were further studied by Santorelli et al. [62] in a larger series of 56 sporadic IBM patients. Multiple mtDNA deletions were found in 73% of patients, and the presence of deletions correlated with morphological evidence of ragged-red, COX-negative fibers, and with defects of complex I and IV of the electron chain.

In 1997, Moslemi et al. [63] sequenced for the first time multiple mtDNA deletions in muscle from four patients with IBM, identifying 33 different deletions. There was a marked predominance of deletions breakpoints in certain regions of mtDNA, like those described in other conditions with multiple deletions, such as autosomal dominant progressive external ophthalmoplegia (adPEO) and normal aging, but different from those described in other conditions due to single deletions such as Kearns-Sayre syndrome and sporadic progressive external ophthalmoplegia [63].

To test the hypothesis of energy metabolism's deficit, Lodi et al. [64] used ^{31}P magnetic resonance spectroscopy (^{31}P-MRS) to assess in vivo skeletal muscle mitochondrial function in the calf muscles of twelve patients with sporadic IBM. On muscle biopsy, 8/12 (66%) patients showed ragged red and/or COX-negative fibers, and in 11/12 (91%) multiple mtDNA deletions were found. The analysis with^{31}P-MRS at resting confirmed the involvement of calf muscle in all patients, disclosing abnormalities in metabolite ratios. However, muscle oxidative metabolism after exercise was normal, as maximum rates of

mitochondrial ATP production and post-exercise ADP recovery rates were within the normal range. Based on these observations, the Authors concluded that, in the pathogenesis of sporadic IBM, mitochondrial abnormalities were a secondary process rather than a *primum movens* [64].

This concept was later resumed by Horvath et al. [65] which characterized molecular defects in mtDNA in the skeletal muscle of patients with familial and sporadic IBM. COX-negative fibers were associated with mtDNA deletions, depletion, and other abnormalities. However, in COX-negative fibers, there was no consistent correlation with markers of inflammation, regeneration, or with the characteristic nuclear pathology. Then, the Authors concluded that microscopically undetectable abnormalities in IBM simply accelerated the accumulation of somatic mtDNA abnormalities occurring in normal aging muscle [65].

Since pathological similarities were observed between IBM and Alzheimer's disease, Kok et al. [66] investigated whether any of mtDNA mutations previously reported in Alzheimer's disease were increased in frequency in IBM, with negative results. Moreover, they also analyzed the evolutionary relationship between the mitochondrial genomes of unrelated individuals by comparing of their D-loop region, both in Alzheimer's disease and sporadic IBM. The phylogenetic analysis showed that the 4336G and 4580A variants cluster together in their respective group. A group of patients with IBM also clustered together on a separate branch of the phylogenetic tree. Closer investigation of this group revealed a common polymorphism at nucleotide position 16311C, suggesting a corresponding mutation in the coding region of mtDNA. All the patients with the 16311C variant, were HLA-DR3 positive [66].

COX-deficient muscle fibers and somatic mtDNA deletions were also found in a family with autosomal dominant hereditary IBM, suggesting similarities with sporadic IBM [67].

Another study about hereditary IBM was performed by Eisenberg et al. [68]. In this work, the Authors analyzed the genomic expression patterns of muscle specimens from ten hereditary IBM patients carrying the M712T Persian Jewish founder mutation and presenting mild histological changes. Compared to controls, the hereditary IBM specific transcriptome consisted of 374 differentially expressed genes, of which 18,6% encoded for protein implicated in various mitochondrial processes, revealing mitochondria pathways dysregulation. Moreover, mitochondrial morphological analysis showed a high degree of mitochondrial branching [68].

In 2014, Joshi et al. [69] investigated the functional relevance of mitochondrial abnormalities in ten patients with sporadic IBM. They studied oxygen desaturation and lactate accumulation during exercise and mitochondrial changes (COX-deficient fibers, biochemical activity of respiratory chain complexes, multiple mtDNA deletions). Compared to controls, sporadic IBM patients had significantly reduced oxygen saturation and elevated serum lactate levels during exercise. The percentage of COX-negative fibers was increased, and five patients had multiple mtDNA deletions, whereas 33% of patients showed an increased citrate synthase content and decrease activities of complex IV. These observations suggested a role of mitochondrial abnormalities in fatigue and exertion-induced symptoms [69].

In the same year, Rygiel et al. [70] examined the prevalence of respiratory mitochondrial deficiency and its relationship with inflammation and atrophy in muscle biopsies from 16 patients with sporadic IBM. Respiratory-deficient fibers were identified at different stages of mitochondrial dysfunction, with downregulated expression of complex I, being the initial feature. mtDNA rearrangements were detected in most individual respiratory-deficient fibers, and there was a strong correlation between number of T lymphocytes and macrophages (marker of inflammation's severity) and the abundance of respiratory-deficient fibers. Moreover, respiratory-deficient fibers were more likely to be atrophic compared with respiratory-normal fibers. The Authors proposed a role for inflammatory cells in the initiation of mtDNA damage, with further accumulation causing respiratory disfunction, atrophy and ultimately fibers degeneration [70].

Shabrokh et al. [71] explored the pathways regulating mitochondrial fusion and fission and regulators of mitochondrial biogenesis and autophagy in skeletal muscle from patients with Alzheimer's disease and sporadic IBM compared with healthy elderly subjects. Patients with sporadic IBM (and Alzheimer's disease) had reduced mitofusin 2 and optic atrophy protein 1, both responsible for mitochondrial fusion. Amyloid βprecursor protein mRNA was higher in sporadic IBM patients only compared to Alzheimer's disease and control group. Furthermore, in sporadic IBM patients, total and phosphorylated AMP-activated protein kinase (AMPK)content, an upstream regulator of mitochondrial dynamics and biogenesis [71].

Lindgren et al. [72] investigated if IBM was associated with sequence variants in genes encoding for proteins involved in mtDNA maintenance. In 26 IBM patients, the Authors studied both mitochondrial genes, such as DNA polymerase gamma gene (POLG), and nuclear genes like Twinkle (C10orf2), DNA2, MGEM1 (earlier C20orf72), POLG2, OPA1, TYMP, RRM2B and SLC25A4 (ANT1). Previously unreported variants in POLG and C10orf2 were found, as well a higher frequency of an RRM2B variant. Moreover, POLG variants were more common in patients with many COX-deficient fibers [72].

In 2016 Rygiel et al. [73] provided an extended spectrum of mtDNA rearrangements, using a complementary set of sensitive assays in individual cells from patients with sporadic IBM. They identified large-scale mtDNA deletions in individual muscle fibers, and two or more deletions were accumulated in 20% of COX-deficient fibers. Most deletions removed only the major arc of mtDNA but about 10% of deletions involved the minor arc, thus removing the origin light strand replication (O_L) and a variable number of genes. Moreover, some mtDNA molecules contained two deletion sites, and there was evidence of mitochondrial genome duplications allowing replication and clonal expansion of these complex rearranged molecules [73].

In the same year, Catalàn-Garcìa et al. [74] studied mitochondrial dysfunction in 30 sporadic IBM patients, not only in muscle, but also in peripheral blood mononuclear cells. They observed depletion of mtDNA in muscle, whereas peripheral blood mononuclear cells showed deregulated expression of mitochondrial proteins in oxidative phosphorylation. In both muscle and peripheral blood mononuclear cells, mitochondrial respiratory chain (MRC) complex IV/citrate synthase activity was significantly decreased. More severe mtDNA depletion was demonstrated in patients with mtDNA deletions, along with deregulation of mitochondrial fusion proteins [74].

In 2018, Buzkova et al. [75] investigated metabolomes of six sporadic IBM patients. Metabolomes of patients with IBM and other mitochondrial myopathies and ataxia clustered separately from controls and non-mitochondrial neuromuscular diseases. IBM showed transulfuration pathway changes (like other mitochondrial disorders), creatine and niacinamide depletion (like non mitochondrial neuromuscular diseases and infantile-onset spinocerebellar ataxia). This omics approach led the Authors to propose nicotinamide riboside and creatine as treatment targets in IBM [75].

In a chloroquine-induced sporadic IBM rat model, Koo et al. [76] demonstrated that mitochondrial quality control and dynamics were improved by resistance exercise, via eliminating CQ-induced beta amyloid accumulation, which resulted in increased mitochondrial biogenesis and mitophagy, decreased formation of rimmed vacuoles and mitochondrial mediated apoptosis, and elevated mitochondrial oxidative capacity. This latter result was due to the up regulation of superoxide dismutase 2, catalase and citrate synthase via activating sirtuin 3 (SIRT3) signaling [76].

Since mtDNA deletions were not found in all sporadic IBM patients, Bhatt et al. [77] investigated if the absence of qualitative abnormalities such as deletions reflected the presence of other change in the multicopy mtDNA genome, like quantitative change of mtDNA depletion. In nine sporadic IBM patients, the Authors observed that muscle contained on average 67% less mtDNA than healthy controls, whereas deletions were present only in four patients [77].

In 2020, Oikawa et al. [78] demonstrated a significant elevation of a mitochondrial disease biomarker, growth differential factor 15 (GDF-15) in sporadic IBM patients' serum and found mitochondrial dysfunction in both patients' myoblasts and skin fibroblasts (decreased ATP production, reduced mitochondrial size and dynamics). Afterwards, the Authors made an experiment with a mitochondria-homing drug, mitochonic acid-5 (MA-5), which they previously demonstrated to increase the cellular ATP levels and reduce mitochondrial ROS (mtROS) production, protecting patients with mitochondrial dysfunction from fibroblast death, and prolonging the survival of a mouse model of mitochondrial disease. Addiction of mitochonic acid-5 to sporadic IBM myoblasts and skin fibroblast improved cell survival and mitochondrial morphology and dynamics with increased ATP. Moreover, the observed reduction in Opa1 and Drp1 gene expression (responsible for mitochondrial fusion/fission) was also reversed by mitochonic acid 5. These observations suggested the potential of mitochonic acid-5 for sporadic IBM therapy [78].

One year later, Hedberg-Oldforset al. [79] investigated in detail the somatic mtDNA variants (deletions, duplications, and single nucleotide variants SNVs) and mtDNA copy numbers in muscle samples from IBM patients and age-matched controls by whole genome sequencing (WGS). In IBM muscles, the Authors observed reduced mtDNA copy numbers, higher levels of large mtDNA deletions and duplications, and more frequent single nucleotide variants, compared to controls. Deletions and duplications mainly localized in 3 mtDNA regions (m.534–4429, m.6330–13,993, and m.8636–16,072). These results indicated an accelerated mitochondrial muscle aging in IBM, compared with normal muscles [79].

2.4. The effects of physical exercise and training

In 1997, Spector et al. [80] studied the effects of a 12-week progressive resistance strength training program in weakened muscles of five patients with sporadic IBM. After 12 weeks, the values of repetition maximum improved in the least weakened muscles, 25–120% from baseline. However, this dynamic effect was not captured by Medical Research Council (MRC) Scale or isometric muscle strength measurements. Repeated muscle biopsies remained unchanged, as well as serum creatine kinase levels and B, T and NK-cell subsets, and muscle size. Based on these observations, the Authors concluded that progressive resistance training in IBM could lead to gains in dynamic strength of the least weakened muscles without causing muscle fatigue/injury or serological, histological, immunological abnormalities [80].

A similar study was conducted by Arnardottir et al. [81] in 2003. They evaluated the effects of physical exercise in seven patients with sporadic IBM, through a home exercise program of five days a week over a 12-week period. After 12 weeks of training, strength was not significantly improved, however muscle function did not deteriorate. Creatine kinase levels were unaffected. Histopathology of repeated muscle biopsies was unchanged and there were no signs of increased muscle inflammation or of expression of cytokines and adhesion molecules. A significant decrease was found in the areas previously positively stained for EN-4 (a marker for endothelial cells), probably expression of the trend for an increase in fiber size after the training leading to a lower capillary density (no changes observed in capillary diameter). Then, the exercise program was not harmful to the muscles regarding muscle inflammation and function, probably allowing to prevent loss of muscle strength due to disease and/or inactivity [81].

Some years later, Johnson et al. [82] tested for muscle strength of seven patients with sporadic IBM before and after a 16-week, patient-specific, home-based exercise program involving mild, daily, functional exercise. They observed a significant improvement in isometric strength of all muscle groups tested (maximally in the hip and flexor muscles), in walking and stair climbing times.

With the addiction of aerobic exercise (cycling 3 times/week on alternative days), aerobic capacity of the group increased significantly by 38% and significant strength improvements were observed in four of the muscle groups tested [83].

In both studies, the exercise program was well-tolerated, and serum CK levels did not significantly change [82,83].

In 2010, Gualano et al. [84] studied the potential applicability of vascular occlusion during resistance training in a patient with IBM. They started from the assumption that the ideal type of exercise for IBM patients should increase muscle strength and cross-sectional area while minimizing exercise intensity, because of the enhancing effect on inflammatory response exerted by high-intensity exercises. The patient underwent moderate-intensity resistance training with vascular occlusion for 12 weeks. Vascular occlusion was obtained using pressure cuffs around muscles during exercise. After the 12-week training program, the patient's leg presses one-repetition maximum, balance and mobility function, and thigh cross-sectional area increased; all Short Form Health Survey 36 (SF-36) Questionnaire subscales demonstrated improvements. Repeated muscle biopsies showed increased mRNA expression of mechano growth factor (MGF), suggesting stimulation of muscle protein synthesis leading to muscle hypertrophy, whereas the decrease in atrogin-1 mRNA expression indicated a decline in muscle proteolysis. The mRNA levels of muscle RING finger-1 (MuRF-1) and mammalian target of rapamycin (mTOR), involved in atrogin-1 and mechano growth factor pathway, respectively, were only slightly altered. Importantly, the exercise program did not induce disease flare and serum creatine kinase remained within normal levels [84].

In the same patient, the Authors also investigated the effects of blood flow restricted resistance training on the expression of genes related to myostatin (MSTN) signaling pathway, whose downregulation was reported to be associated with overload-induced increments in muscle hypertrophy and strength (thereby preventing muscle wasting). After the 12-week blood flow restricted resistance training program, repeated muscle biopsies showed a moderate decrease in myostatin mRNA expression, whereas gene expression of the myostatin inhibitors such as follistatin and follistatin-like 3 was up-regulated, probably revealing the molecular mechanisms involved in the muscle hypertrophy following blood flow restricted resistance training [85].

Further observations about beneficial effects of blood flow restricted resistance training came in 2015 from the study by Jorgensen et al. [86]. In this work, the Authors demonstrated that blood flow restricted resistance training was well tolerated by a patient with sporadic IBM and led to substantial improvement in mechanical muscle functions and gait speed.

Three years later, the same Authors conducted a randomized controlled trial about blood flow restricted resistance training in 22 patients with sporadic IBM. In this trial, 12-weeks blood flow restricted resistance training did not improve self-reported or objective physical function. However, blood flow restricted resistance training had a preventing-retaining effect on the disease-related decline in leg muscle strength, whereas leg muscle strength decreased in controls [87].

Another RCT was performed by Jensen et al. [88] in 2019 to investigate the effect of blood flow restricted resistance training on adaptative and innate immune response of the affected muscles in 22 sporadic IBM patients. After 12-weeks blood flow restricted resistance training program, muscle biopsies showed important findings: myocellular infiltration of CD3-/CD8+ NK cells significantly increased after blood flow restricted resistance training, whereas no changes were observed in the control group; pronounced infiltration of M1 pro-inflammatory (CD68+/CD206-) and M2 anti-inflammatory (CD68+/CD206+) macrophages, observed at baseline, persisted after the training, as well as T cell infiltration did not undergo significant changes. These observations suggested that blood flow restricted resistance training evoked an amplified immune response in sporadic IBM muscle through upregulation of NK cells, however without intensifying inflammatory activity (no changes for macrophages and T cells infiltration) [88].

Respiratory complications frequently occurred in IBM, due to weakness/fatigue of respiratory muscles. In this regard, Cordeiro de

Souza et al. [89] reported the case of a patient who developed respiratory failure requiring intubation and mechanical ventilation, despite immunosuppressive treatment. The use of an inspiratory muscle training program, using an isokinetic electronic load device, allowed a successful weaning in one week and hospital discharge after 2 subsequent weeks [89].

Kwon et al. [90] studied the effects of long-term exercise training in rat skeletal muscle of chloroquine-induced sporadic IBM. They demonstrated that sporadic IBM induced by chloroquine treatment resulted in muscle degeneration via impaired autophagy, and that resistance exercise training improved movable loading activity, probably providing protection against sporadic IBM by enhancing the autophagy flux through p62 protein [88].

Another study on resistance exercise and autophagy was conducted by Jeong et al. [91] in a sporadic IBM rat model induced by chloroquine. In this work, the effects of chloroquine and resistance exercise differed, depending on myofibres' characteristics. In soleus muscles, chloroquine induced abnormal autophagy, with increased generation of rimmed vacuoles and accumulation of beta amyloid, resulting in atrophy. In the same muscles, resistance exercise generated rimmed vacuoles, decreased amyloid deposition, and improved autophagy without generating hypertrophy, thus reducing the atrophy signal transduction, and then decreasing atrophy compared to chloroquine control group. In *flexor hallucis longus* muscles, despite no direct effect of chloroquine, loss of mass was observed, due to reduced activity or increased inflammatory response, and resistance exercise increased the hypertrophy signal, resulting in reduced autophagy and atrophy [91].

The same group, starting from the assumption that resistance exercise could prevent muscle impairment by improving mitochondrial function via eliminating beta amyloid accumulation in sporadic IBM muscle, used again a chloroquine-induced sporadic IBM rat model to verify their hypothesis. As results, the Authors observed that resistance exercise markedly inhibited *soleus* muscle atrophy and muscle damage, and reduced chloroquine-induced beta amyloid accumulation, resulting in decreased formation of rimmed vacuoles and mitochondrial-mediated apoptosis. Moreover, the decrease beta amyloid accumulation (induced by resistance exercise) improved both mitochondrial quality control (through increased mitochondrial biogenesis and mitophagy) mitochondrial dynamics, and elevated mitochondrial oxidative capacity by upregulating superoxide dismutase 2, catalase and citrate synthase via activating sirtuin 3 (SIRT3) signaling [76].

In 2022, a double-blind, placebo-controlled, cross-over trial by Coudert et al. [92] investigated whether 12 weeks of testosterone supplementation combined with a 26-weeks personalized progressive exercise training program in 14 men with IBM was more effective in improving the peripheral immune manifestations associated with the disease than exercise alone. Testosterone supplementation resulted in modest yet significant count reduction in the classical monocyte subset and eosinophils, while testosterone independent immunoregulatory effects attributed to exercise included altered proportions of some monocytes, T and B-cell subset, and reduced pro-inflammatory cytokines (IL-12, IL-17, TNF-α, MIP-1β and sICAM-1) [92].

Finally, Li et al. [93] recently developed a feeder-supported in vitro exercise-model using human satellite cells from patients with sporadic IBM, potentially utilizable for evaluating exercise-dependent intrinsic and pathogenic properties of patients' muscle cells [93].

3. Oxidative stress, microRNAs, and idiopathic inflammatory myopathies

The influence of epigenetic control exerted on the immune system by non-coding genetic material is a novel component of the interaction between oxidative stress and IIM. Short, non-coding, single-stranded RNAs called microRNAs (miRNAs) target certain mRNAs for translational inhibition or degradation. Thus, miRNAs control several cellular functions and pathways and affect the expression of genes in different pathologies [94,95].

Several autoimmune diseases, including IIM, have been reported to have dysregulated miRNAs that control the immune system, and IIM has also been found to have dysregulated miRNAs that are crucial for the growth and maintenance of skeletal muscles [96–98], and autoimmunity seems closely correlated with the upregulation of immune-related miRNAs in muscle [99]. Thus, the suppression of muscle regeneration is linked to the downregulation of miRNAs such miRNA-1 and miRNA-206 [100,101]. These miRNAs are also a part of the newly discovered myomiRs group of miRNAs [102,103].

Recently, understanding of inflammation, muscle weakness/wasting, and extra-muscular organ involvement in IIM has improved due to the identification of dysregulated miRNAs [104]. By modifying in-vitro settings and observing relevant miRNA levels, or, alternatively, by adjusting miRNA levels and observing potential targets, disease mechanisms have been investigated. Several research have examined the function of miRNAs that can affect oxidative stress in this situation. For instance, increased concentrations of cytokines that can reduce oxidative stress seem to suppress myogenic miRNAs in muscle.

There are intricate connections between oxidative stress, miRNAs, and inflammatory myopathies, according to other studies. Several diseases exhibit dysregulation of the carcinogenic miRNA, miRNA-96-5p, which is also involved in the regulation of oxidative stress [105]. Adenosine is changed into adenosine monophosphate and adenosine diphosphate by the enzyme adenosine kinase (ADK). A lack of ADK and overexpression of miRNA-96-5p have both been linked to mitochondrial dysfunction [106,107]. The myositis-associated weakness may be related to altered reactive oxygen species generation brought on by mitochondrial malfunction [108]. So, if miRNA-96-5p targeting of ADK is verified, this would be a fruitful area of investigation in myositis. In fact, miRNA-96-5p was shown to be considerably overexpressed in polymyositis and dermatomyositis [109]. MiRNA-96-5p upregulation was reproduced by RTqPCR, and anticipated mRNA targets (ADK, CD28, and SLC4A10) were downregulated, and considerable downregulation of ADK was seen after miRNA-96-5p mimic transfection of a human skeletal muscle cell line.

Furthermore, according to a prior study, catalpol can suppress p66shc/A-96-5p, which reduces oxidative stress [110]. While it is well known that overexpression of miRNA-196a-5p reduced H2O2-induced oxidative damage in SRA01/04 cells, miRNA-193b-3p and miRNA-196a-5p expression levels in this investigation were negatively associated in polymyositis and dermatomyositis, respectively [111].

According to a different experiment, myomiRs, particularly miRNA-1, miRNA133a, miRNA-208b, miRNA-486, and miRNA-499, function in a network and are connected to the emergence of PM [112]. Some of these miRNAs are redox-sensitive miRNAs, though.

Finally, the hormone adiponectin (ApN) has become a master regulator of immune response and inflammation in a variety of tissues, including skeletal muscle. A study investigated whether microRNAs controlled by adiponectin might be new mechanisms for regulating muscle inflammation [113]. According to the findings, miRNA-711 is a leading candidate to mediate adiponectin action. As a result, although animals overexpressing adiponectin showed elevated miRNA-711 levels, adiponectin-knockout mice showed lower muscle expression of miRNA-711 along with increased inflammation/oxidative stress indicators. The adiponectin gene was electrotransferred into the muscle of adiponectin-knockout mice, which increased miRNA-711 levels while lowering oxidative stress and inflammation. Similar results were achieved using human primary myotubes or murine C2C12 cells that had been treated with adiponectin.

Overexpression of MiRNA-711 suppressed the activity of NF-B and downstream pro-inflammatory cytokines by downregulating numerous Toll-like receptor-4 pathway components. Blocking MiRNA-711 had the opposite results. Additionally, pre-miRNA-711 muscle electrotransfer replicated in vivo the anti-inflammatory benefits seen in vitro. As a result, miRNA-711, which adiponectin upregulates, inhibits TLR4

signaling and so serves as a key mediator of adiponectin's anti-inflammatory effects [113]. This new miRNA and the genes that it targets may provide unique therapeutic opportunities for reducing muscular inflammation [114]. Therefore, understanding the function of miRNAs in IIM will be crucial in the years to come. If these miRNAs are to serve as biomarkers or pharmacological targets, additional research will be required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

MGD and SG were responsible for the study's conception and design. EA, MAP, MFC, and AA reviewed the studies and wrote the first draft. EA drawn the figure. All Authors contributed to analyses of the references. MGD, EA, AA, and SG revised the manuscript critically for intellectual content. All authors gave their final approval of the version of the manuscript to be published.

Data availability

Data will be made available on request.

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