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Review Article

Exercise-induced mitochondrial dysfunction: a myth or reality?

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Abstract

Beneficial effects of physical activity on mitochondrial health are well substantiated in the scientific literature, with regular exercise improving mitochondrial quality and quantity in normal healthy population, and in cardiometabolic and neurodegenerative disorders and aging. However, several recent studies questioned this paradigm, suggesting that extremely heavy or exhaustive exercise fosters mitochondrial disturbances that could permanently damage its function in health and disease. Exercise-induced mitochondrial dysfunction (EIMD) might be a key proxy for negative outcomes of exhaustive exercise, being a pathophysiological substrate of heart abnormalities, chronic fatigue syndrome (CFS) or muscle degeneration. Here, we overview possible factors that mediate negative effects of exhaustive exercise on mitochondrial function and structure, and put forward alternative solutions for the management of EIMD.

Key words: aging, athletes, DNA deletion, exhaustive exercise, peroxisome proliferator-activated receptor γ co-activator 1-α (PGC-1α), reactive oxygen species.

INTRODUCTION

Mitochondria have long been recognized as a key element of cellular viability [1], with the organelle now confirmed to be involved in a plethora of fundamental life processes. These organelles are the main cellular sources of energy through oxidative phosphorylation, important regulators of redox production and signalling, modulators of calcium homoeostasis, haem biosynthesis and amino acids utilization and major players in the control of stress responses and apoptotic cell death [2]. Preserved mitochondrial function seems to be the most important determinant of long lifespan [3], whereas its dysfunction accompanies or triggers myopathies, neurodegenerative and cardiometabolic disorders, cancer and aging [4]. Thus, the organelle becomes an important target for different pharmacological and non-pharmacological interventions to tackle mitochondrial dysfunction [5], with exercise often suggested as a therapy of choice. Many studies have reported beneficial effects of physical exercise on mitochondrial content and function [6–8], with regular exercise alleviating signs and symptoms of mitochondrial dysfunction in aging, diabetes and brain disorders [9–11]. However, several studies questioned this paradigm, suggesting that extremely heavy or prolonged exercise might actually induce mitochondrial disturbances that could permanently impair its function. St Clair Gibson et al. [12] reported a case of an apparently healthy top-level athlete who developed an irreversible mitochondrial dysfunction after years of exhaustive training. In addition, several studies in rodents suggested that exhaustive exercise might induce an inhibition of mitochondrial phosphorylative activity [13], and hard-to-recover mtDNA deletions and cell death [14]. It appears that exercise strongly affects mitochondrial structure and function, yet the direction and the degree of change are open to the debate. In this paper, I will discuss possible factors that mediate negative effects of exercise on mitochondrial function, and put forward alternative solutions for the management of exercise-induced mitochondrial damage.

Beneficial effects of exercise on mitochondrial function

One of the classical responses to exercise is an increase in the number and function of mitochondria, with improved mitochondrial quality and quantity closely related to several of the positive health effects reported after training [15]. After the transient decrease in mitochondrial performance seen immediately after an exercise session [16], mitochondrial biogenesis amplifies, with

Abbreviations: BDNF, brain-derived neurotrophic factor; CFS, chronic fatigue syndrome; COX, cyclooxygenase; CS, citrate synthase; DRP, dynamin-related protein; DRP1, dynamin-related protein 1; EIMD, exercise-induced mitochondrial dysfunction; HSP 70, heat shock protein; LV, left ventricle; MDA, malondialdehyde; $\Delta\Psi$mt, mitochondrial membrane potential; mtMTP, mitochondrial trifunctional protein; MTP, mitochondrial permeability transition pore; nDNA, nuclear DNA; NRF, nuclear respiratory factor; OPA1, optic atrophy 1; PGC-1α, peroxisome proliferator-activated receptor γ co-activator 1-α; RNS, reactive nitrogen species; ROS, reactive oxidative species.

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favourable changes in mitochondria volume and number [17]. The organelles grow in size and density, mitochondrial fuel utilization shifts toward an increased use of lipids as a substrate source, and the mitochondrial enzyme capacity expands [18]. Consequently, oxidative capacity and exercise performance increase. It seems that regular exercise positively influences the expression of peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α), a key regulator of mitochondrial biogenesis and function [19]. Endurance exercise appears to be particularly effective in this manner, with even a single 60 min aerobic exercise inducing gene expression changes that positively affect mitochondria in both exercising and non-exercising muscle of healthy men [20]. Favourable mitochondrial adaptations after regular exercise are also reported in clinical patients with different disorders [21,22] or aging population [23]. Even severely damaged mitochondria improve their function after regular aerobic exercise [24]. However, much less is known about the dose–response relationship between favourable mitochondrial changes and the intensity/volume of exercise. Several studies advanced high-intensity exercise as an effective model for improving mitochondrial biogenesis and function [25,26]. On the other hand, a recent study reported that PGC-1α mRNA expression was negatively correlated with exercise intensity [27], suggesting that transcriptional activity of the mitochondrial biogenesis signalling cascade is exercise intensity-sensitive. Optimized exercise load could be of critical importance for specific mitochondrial adaptations, yet whether different intensities demonstrate biologically different mechanisms involved in 'acclimatization to exercise' remains currently unknown.

Mitochondrial dysfunction induced by exercise

The term 'dysfunctional mitochondria' is widely used in cell biology and bioenergetics research and clinical medicine. However, its precise definition is rather difficult, and depends on whether dysfunction is to be determined with isolated organelle, intact cells or *in vivo*, and which biomarkers (clinical or experimental) are available for assessing mitochondrial performances. Usually, mitochondrial dysfunction is defined as an impaired ability of the mitochondria to make ATP, the major energy carrier in the cell, appropriately in response to energy demands, although abnormality in other processes governed by mitochondria can be termed mitochondrial dysfunction as well [28]. Diagnostic strategies for mitochondrial disorders/dysfunction require multi-disciplinary evaluation, and rely on a combination of clinical observations, laboratory evaluation, brain imaging and skeletal muscle biopsies, with no single 'golden standard' test currently available to diagnose mitochondrial dysfunction [29]. Mitochondrial dysfunction occurs early and acts causally in many diseases and conditions [30], with several factors having been identified to induce this condition, and disturb energy metabolism or free-radical generation in the body [31–33]. Understanding its aetiology could help to identify vulnerability traits and avoid provoking agents, including different drugs and toxic agents or other mitochondria-targeted damaging interventions. Previously, there has been speculation that excessive endurance exercise may be deleterious to various biological systems and subcellular structures [34], in which mitochondrial dysfunction might play a role [35].

About 50 years ago, Laguens et al. [36] were first to report severe modifications of mitochondrial structure in myocardium of dogs submitted to exhaustive exercise, with frequently observed giant mitochondria with partial vacuolization of the matrix and disruption of the cristae. Gollnick et al. [37–39] evaluated the fine structure of heart and skeletal muscle following exhaustive exercise in the series of seminal studies conducted in rats and humans. Among other findings, authors reported mitochondrial swelling in rats that had completed approximately 450 h of exhaustive swimming, with changes largely reversed by 15–18 h recovery period. However, some mitochondria were grossly swollen with badly disrupted and degenerated cristae (most prominent in the myocardial mitochondria), with metabolic capacity of dysfunctional organelles seeming to be adversely altered after prolonged severe exercise. These observations suggest that exhaustive exercise might markedly impair mitochondrial function and/or structure, at least in a given area or tissue. Gohil et al. [40] confirmed the above findings, reporting exercise-induced decrease in mitochondrial activity in brown adipose tissue of rats subjected to exhaustive running, with mitochondrial oxidative pathways stressed more in untrained rats compared with trained counterparts. In the past 20 years, several studies reported similar detrimental effects of extremely heavy exercise on mitochondrial performance, with permanent or long-term exercise-induced mitochondrial dysfunction (EIMD) found in the brain, skeletal muscle, heart, liver and blood cells of rodents and humans [12,14,35,41–52]. A summary of those studies is presented in Table 1.

Exhaustive exercise seems to negatively affect different markers of mitochondrial health, including a disruption of activity and/or expression of mitochondrial enzymes [cyclooxygenase (COX), citrate synthase (CS), malondialdehyde (MDA)] and mitochondria-related growth factors [PGC-1α, mitogen-activated protein kinase, brain-derived neurotrophic factor (BDNF)], an amplification of mtDNA deletions and mitochondrial apoptotic factors expression [dynamin-related proteins (DRPs), transcription factor A], a reduction in mitochondrial membrane potential ($\Delta\Psi$mt), and enhanced production of mitochondrial reactive oxidative species (ROS). On the other hand, several biomarkers of mitochondrial function in human studies are hard to interpret, with a drop in leucocyte mitochondrial trifunctional protein (mtMTP), or an increase in NADH oxidase system of muscle mitochondria not necessarily indicating mitochondrial damage after exhaustive exercise. Ultimately, strenuous exercise induces severe ultrastructural changes in the organelle, including uneven mitochondrial distribution with subsarcolemmal mitochondrial aggregation, and high prevalence of large and swollen mitochondria with dense matrices and coarse or abnormal cristae. EIMD appears in both males and females submitted to different modes of exercise to exhaustion (e.g. running, cycling, swimming) in both acute and chronic exercise model. At the moment, no clear guidelines have been established concerning diagnostic criteria for EIMD. It seems that the severity (and implied irreversibility) of this phenomenon might be a key aspect that should be used to discriminate between transient decrease in mitochondrial performance and more severe EIMD. This could be related to critical changes in mtDNA or nuclear DNA (nDNA) (e.g. large-scale deletions induced by exhaustive exercise) that permanently alter

Table 1 Studies evaluating mitochondrial dysfunction after exhaustive exercise

Reference	Subjects	Exercise regimen	Main outcomes for mitochondrial function
[12]	Trained man (n=1)	Multi-year endurance training approximately 100 km/week	⇑ Percentage of abnormal mitochondria in vastus lateralis
			⇑ Subsarcolemmal mitochondrial aggregation
[14]	Trained male rats (n=32)	Acute exercise model	⇑ mtDNA4834 deletion in LV tissue
		Run to exhaustion (30 m/min at 10% inclination)	⇑ Apoptosis index for Bcl-2-associated X protein (Bax/Bcl-2) ratio of LV, cleaved caspase-3, poly (ADP-ribose) polymerase (PARP), cytochrome c
			⇑ Apoptosis-induced DNA strand breaks in cardiac myocytes
[35]	Untrained 8-week old rats (n=40)	8-week exercise on a treadmill (6 days/week, 60 min at 20 m/min, 5° grade)	⇓ PGC-1α and complex 1 subunit expression in the skeletal muscle
		Sprint to exhaustion at follow up (30 m/min at 5° grade)	⇑ mRNA of mitochondrial transcription factor A (mtTFAM)
			⇑ Expression of mt DRP1
[41]	Untrained 8-week old male rats (n=9)	Acute exercise model	⇑ Large-scale deletion (7052 bp) of mtDNA
		Sprint to exhaustion (40 m/min)	⇑ Mitochondrial abnormalities in the soleus muscle
[42]	Young men (n=6)	6-week exercise training (trained group) compared with sedentary group	⇓ NAD-linked activities of pyruvate dehydrogenase (PDH), α-oxoglutamate dehydrogenase (GDH) in vastus lateralis muscle
		Five 1 min cycling bouts (90 rpm) to exhaustion	⇑ Exo-NADH oxidase, α-glycerophosphate dehydrogenase
[43]	Female rats (n=24)	Acute exercise model	⇑ Oxidizing pyruvate and succinate in gastrocnemius of trained rats
		Running to exhaustion (26 m/min at 15° slope)	⇓ Oxidizing pyruvate, 2-oxolutare in liver of untrained animals
[44]	Trained men (n=12)	Acute exercise model (running on a treadmill)	⇓ Leucocyte mtMTP after exhaustive exercise
		30 min at 35–85% maximal oxygen uptake (VO_{2max}) for three consecutive days	
[45]	Adult male mice (n=72)	8-week exercise on a treadmill	⇓ Brain cortex COX activity
		5 days/week, 45 min/day at 13.5–16.5 m/min	⇓ BDNF
[46]	Trained men (n=12)	3 days of high-intensity exercise	⇓ Leucocyte MTP 24 and 48 h post-exercise
		30 min/day at 85% VO_{2max}	
[47]	24-week old rats (n=49)	1 week exercise on a treadmill (10 min at 10 m/min at 5° slope)	⇑ mt MDA in soleus and gastrocnemius muscle
		Sprint to exhaustion at follow up (25 m/min at 5° grade)	⇓ mt GSH/GSSG
[48]	Young and old mice (n=60)	5-day exercise on a treadmill (approximately 50 min/day)	⇑ Mitochondrial ROS production in old high-intensity group
		High- compared with low-intensity running (8.8–23.8 m/min until exhaustion)	⇑ mtDNA/nDNA ratio, CS and COX activity in young high-intensity group
			⇓ Mitochondrial production of ATP (MAPR) in the soleus muscle of high-intensity group
[49]	Untrained 8-week old rats (n=64)	Acute exercise model (incremental treadmill running)	⇓ mt state 3 respiration (ST3) rate in the myocardium of heavily-exercised rats
		Phase 1: 15 min at 8.2 m/min followed by 15 min at 15 m/min, 5° grade	⇓ $\Delta\Psi$mt and mitochondrial ATP synthase activity in heavily-exercised rats
		Phase 2: 19.3 m/min at 10° grade for 15, 60 or 90 min	⇑ Mitochondrial ROS production in heavily-exercised rats
		Phase 3: post-exercise recovery (12, 24, 36 and 48 h)	

Table 1 Conitnued

Reference	Subjects	Exercise regimen	Main outcomes for mitochondrial function
[50]	Rats (n=40)	One-time exhaustive swimming exercise	⇑ Mitochondrial injury
			⇓ Mitochondrial respiratory function
[51]	Young and old men (n=40)	Supramaximal plantar flexion (120% of maximal aerobic power)	⇑ Mitochondrial ATP cost of contraction in old group
			⇔ Peak rate of mitochondrial ATP synthesis
[52]	Untrained men and women (n=16)	Alternate knee extensions every 2 s [40% voluntary maximum isometric force (MVC)] until exhaustion	⇓ Maximal rate of mitochondrial oxidative ATP synthesis

gene expression at the level of transcription and/or translation. Extreme production of mitochondrial ROS and nitrogen species during exhaustive exercise seems to induce exercise-related DNA damage [53], making mtDNA particularly susceptible to oxidative stress, and a pathophysiological target for EIMD. mtDNA seems to have a much higher mutation rate compared with nDNA, since it is readily exposed to ROS damage while lacking protective histones and other DNA repair mechanisms [54]. Therefore, monitoring mtDNA deletions at specific regions (such as ΔmtDNA6829 and ΔmtDNA6992) and post-exercise changes in genetic profiles using ΔΔPCR-based technique [55] might be employed as a novel tool to evaluate EIMD severity and progression. Although ROS-mediated mtDNA alterations could induce EIMD, other mechanisms might be accountable as well (Figure 1).

Enhanced production of ROS and reactive nitrogen species (RNS) during physical exercise occurs as a consequence of oxygen-dependent bioenergetics in mitochondria, with electron transport chain and mitochondrial xanthine oxidase activity recognized as main sources of these compounds [58]. Another exercise-related source of ROS is the inflammatory response to tissue injury (as induced by successive muscular contractions) with neutrophil activation and macrophage infiltration producing large amounts of ROS [59]. Recently, hyperthermia, dehydration and osmotic stress were also identified as unconventional sources of ROS generated during exercise [60], with the effects of exercise on ROS generation seeming to be intensity-dependent. Although mild exercise appears to balance mitochondria-related ROS production and induces favourable ROS-associated adaptations, exhaustive or long-lasting exercise stimulates an overproduction of ROS [61]. Hence, too much ROS could damage subcellular biomolecules, such as lipids, proteins and DNA (for detailed review see reference [58]), and heavily jeopardize mitochondrial function, leading to EIMD.

Although EIMD affects all ages, it seems that being older could be a predisposing factor for EIMD. Aging per se induces profound changes in mitochondrial form and function, including DNA deletions, augmented oxidative stress and impaired mitochondrial bioenergetics [62–65]. When exposed to strenuous exercise, it seems that old subjects more easily develop mitochondrial dysfunction and accelerated senescence. Lee et al. [48] recently evaluated effects of heavy exercise in skeletal muscle of mice at age 2 months (young group) and 24 months (old group), subjected to 5 days exercise regimen (running on a motorized treadmill until exhaustion). Exhaustive exercise in old mice resulted in the decreasing of both fusion (mitofusin-2) and fission (dynamin-1-like) proteins that may contribute to alteration of mitochondrial morphology, and reduced PGC-1α nuclear translocation. Furthermore, there was a 69% increase in interleukin-1β (an important mediator of the inflammatory response) in the old group, whereas exhaustive exercise did not affect this biomarker in young mice. Authors concluded that exhaustive exercise in senescent muscles magnifies mitochondrial damage, being an inappropriate mode of exercise for treating aging and age-related mitochondrial diseases.

Another factor that might determine EIMD susceptibility is a previous training status. EIMD affects both trained and non-trained subjects, yet this phenomenon seems to be more frequent in overtrained population [15,43,66], suggesting a dose–response curve for EIMD. Repetitive exposure to extremely heavy or prolonged exercise activity could induce mitochondrial damage in susceptible individuals that accumulates over time, and eventually becomes chronic and beyond repair, with long-term implications for exercise performance and health [67]. However, no clear exposure–response relationship between exhaustive exercise load (e.g. frequency, intensity, duration and type of exercise) and EIMD has been described so far. However, exercise intensity might play a crucial role in EIMD aetiology, since the expression of stress protein, heat shock protein (HSP 70) that jointly regulates mitochondrial function, is exercise-intensity dependent [68]. Finally, EIMD appears to show tissue-specific responses, with myocardial mitochondria suffering the most from exhaustive exercise, as compared with brain, liver or skeletal muscle mitochondria [43]. This might be due to higher rates of oxygen consumption per milligram of protein in heart mitochondria [69] and consequent hyperproduction of organelle-damaging ROS.

Possible health consequences of EIMD

Although regular physical activity reduces health risks for many diseases, previous studies have documented that exhaustive exercise poses a variety of health hazards even in healthy individuals, a fact that raised concerns about detrimental consequences of such exercise [70]. Besides other possible factors, EIMD might be a key proxy for negative outcomes of exhaustive exercise, being a pathophysiological substrate of heart abnormalities, chronic fatigue and overtraining syndrome or muscle degeneration. Pierce et al. [71] were among the first to demonstrate that strenuous

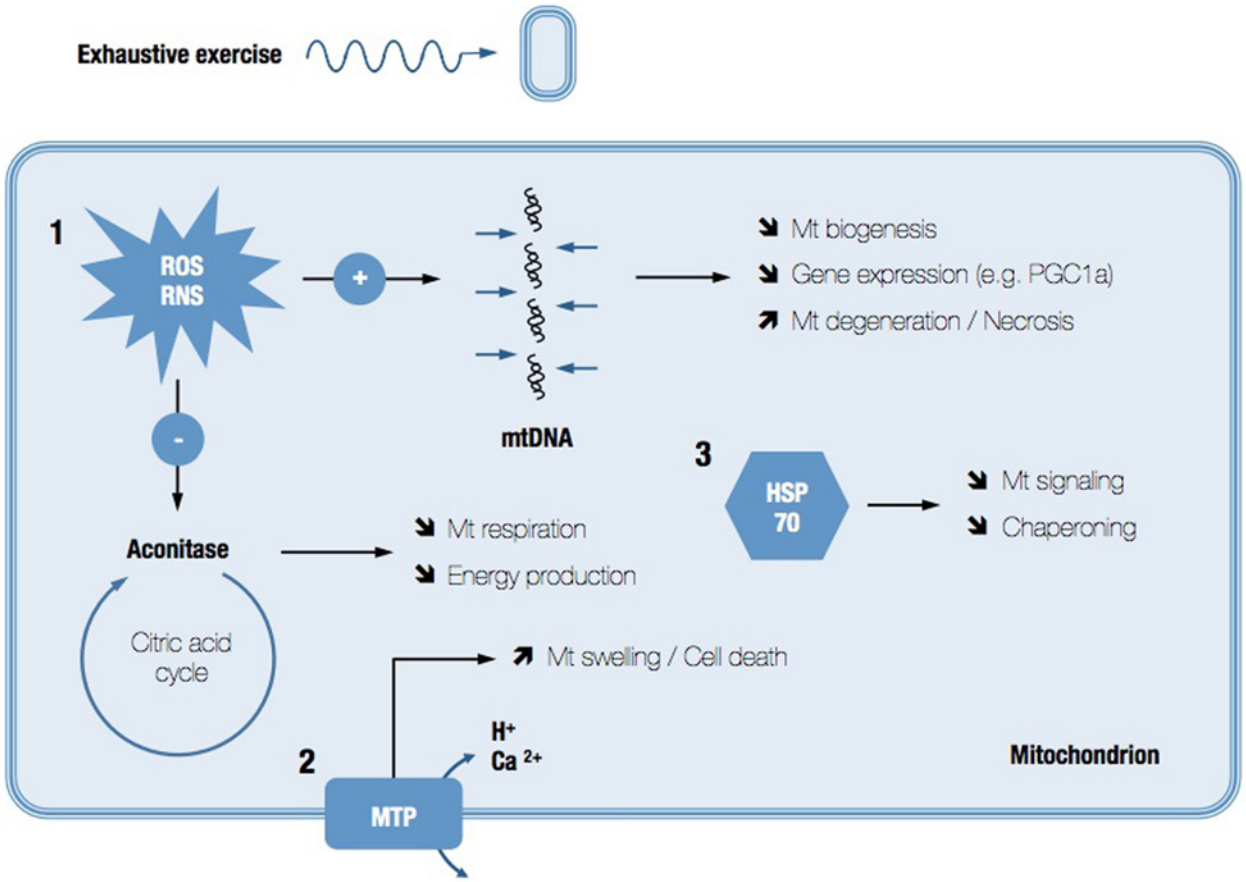

Figure 1 **Possible factors that induce EIMD**

(**1**) Overproduction of ROS and RNS could stimulate (+) medium-to-large deletions of mtDNA that diminish mitochondrial biogenesis and down-regulate gene expression (such as PGC-1α), and result in degeneration of the organelle [53]; or inhibit (−) aconitase, a regulatory enzyme in the citric acid cycle, driving inadequate mitochondrial respiration and energy production [56]. (**2**) Mitochondrial permeability transition pore (MTP) opening might affect calcium and protons flow through inner membrane resulting in mitochondrial swelling and cell death [42]. (**3**) Exercise-induced hyperthermia could induce an overexpression (or misexpression) of 70 kDa HSP 70 that jeopardizes mitochondrial signalling, chaperoning and macromolecular integrity [57].

exercise is capable of producing biochemical changes in myocardial mitochondria (e.g. depressed mitochondrial accumulation of Ca^{2+}) that may adversely affect heart function after consecutive bouts of exhaustive exercise. Pan [72] found tumefied mitochondria in cardiomyocytes of exhaustively exercised rats, with possible arryhthmogenic changes in atrial natriuretic peptide levels and expression. Chang et al. [73] recently evaluated exercise-induced cardiac injury in rats following repeated exhaustive exercise. Authors reported significant mitochondrial alterations, accompanied by ischaemic alterations, cellular damage to cytoskeleton and gap junctions and tissue fibrosis in the cardiac conduction system, with the above mitochondrial disturbances known to induce cardiac arrhythmias [74]. Similarly, Olah et al. [75] reported dysfunctional mitochondria-related cardiac stress in rats forced to swim for 3 h, including a dysregulation of the matrix metalloproteinase system, increased nitro-oxidative stress and sporadic fragmentation of myocardial structure. Clinically relevant disturbances in haemodynamics [e.g. increased end-systolic volume, decreased ejection fraction, impaired contractility and mechanoenergetics of left ventricle (LV) after exercise] accompanied histological changes. No human studies known to the author linked EIMD to cardiac dysfunction yet some arrhythmias in athletic population might have a mitochondrial origin, with mitochondria-targeted antioxidants highlighted as a novel antiarrhythmic therapy [76].

Chronic fatigue syndrome (CFS), a complex medical condition comprising of persistent post-exertional malaise, widespread pain in musculoskeletal system, and mental and physical exhaustion not substantially relieved by rest, is a prevalent disorder with unknown aetiology, affecting up to 5 % of the general population worldwide [77]. Several previous studies suggest that mitochondrial dysfunction has been involved in the pathophysiology of CFS [78–80]. In addition, long-term heavy exercise could induce CFS in athletes [81] or magnify exhaustion in CFS patients [82], suggesting that EIMD might be a cofactor that triggers CFS. Overtraining is another perplexing condition that might be related to EIMD. Usually described as a long-term excessive overload with inadequate recovery that is accompanied by a

decrease in performance [83], overtraining remains difficult to diagnose and manage due to unknown cause. A seminal paper by St Clair Gibson et al. [67] described several cases of mitochondrial pathology in apparently healthy but overtrained top-level athletes, with detrimental changes in skeletal muscle structure and function associated with many years of excessive training and competing. Authors suggested that there may be a finite capacity for muscle regeneration after exhaustive exercise which, when exceeded, initiates overtraining and the deterioration of athletic performance. Accordingly, Wang et al. [84] recently suggested that mitochondrial dysfunction could contribute to the development of muscle disorders, including muscle wasting, muscle atrophy and degeneration. ROS formation and associated oxidative stress in the skeletal muscle are critical to mitochondrial dysfunction which is characterized by down-regulation of optic atrophy 1 (OPA1; a key protein that regulates mitochondrial inner membrane fusion and remodelling) and myosin heavy chain protein loss, eventually leading to significant morphology changes in myotubes and muscle cell degeneration. The role of mitochondria in muscle-damaging exercise was confirmed in another trial [35], with strenuous exercise-induced muscle dysfunction accompanied by increased mitochondrial fission, increased muscle atrophy markers (atrogin-1 and muscle RING-finger protein-1 mRNA) and triggered cell autophagy. Interestingly, augmented mitochondrial fission in damaged myocytes after heavy exercise (as evaluated by an increase in dynamin-related protein 1, DRP1) in this study was similar to DRP1 response found in skeletal muscle after a high-fat diet [85], perhaps suggesting a similar mechanism of mitochondrial dysfunction in exercise-induced model and obesity. However, despite a limited understanding of mechanisms accounting for mitochondria-related muscle disorders, EIMD should be further investigated as a possible pathogenic factor of myocyte damage *in vivo*. Although EIMD is more emphasised in skeletal muscle, Aguiar et al. [45] reported that exhaustive exercise also promotes brain mitochondrial dysfunction, probably due to exercise-induced inhibition of BDNF production in frontal cortex. This might explain cognitive disturbances seen in CFS and overtraining syndrome. However, more mechanistic studies are needed to establish a link between EIMD and long-term health consequences of exhaustive exercise in athletes and clinical population.

Management strategies for EIMD

Besides exercise intervention, that probably represents the key element of prevention and dealing with dysfunctional mitochondria, several mitochondria-targeted agents might be considered to overcome or at least attenuate, EIMD. Supporting mitochondrial bioenergetics and helping mtDNA to repair after exhaustive exercise, and maintaining a high antioxidant capacity to scavenge toxic ROS inside the organelle comprise possible treatment options for mitochondrial dysfunction induced by extremely heavy or prolonged exercise. Antioxidants and allied nutraceuticals are widely discussed in the clinical and nutritional literature (for detailed review see references [86–88]). However, only a limited number of studies evaluated the effectiveness of mitochondria-targeted interventions in EIMD using organelle-specific biomarkers. Ping et al. [50] evaluated protective effects of salidroside, a glucoside of tyrosol found in the plant *Rhodiola rosea*, on mitochondrial dysfunction and cardiomyocyte injury induced by exhaustive swimming exercise in rats. Administration of salidroside (100–300 mg/kg per day for 2 weeks) attenuated myocardium injury and ultrastructural mitochondrial malformations, preserved mitochondrial respiratory function, and counteracted maladaptive gene expression of PGC-1α and nuclear respiratory factors [nuclear respiratory factor 1 (NRF-1) and nuclear respiratory factor 2 (NRF-2)] compared with control group receiving placebo (12 mg/kg per day of 0.9 % NaCl). In another study, Feng et al. [35] reported protective effects of hydroxytyrosol, a natural olive polyphenol, in strenuous exercise-induced muscle and mitochondrial dysfunction with Sprague-Dawley 8-week-old male rats. Hydroxytyrosol treatment (25 mg/kg per day for 8 weeks) inhibited excessive exercise-induced increase in autophagy and mitochondrial fission, and the decrease in PGC-1α expression. In addition, hydroxytyrosol enhanced mitochondrial fusion and mitochondrial complex I and II activities. A recent study by Carfagna et al. [89] investigated effects of microalga *Galdieria sulphuraria* on EIMD elicited by acute strenuous exercise (6 h swimming) in rats. *G. sulphuraria* treatment (10 g/kg per day for 10 days) reduced exercise-increased protein carbonyl content, an indicator of oxidative damage, in mitochondria from heart and muscle of heavily-exercised rats. In addition, Gao et al. [90] reported beneficial effects of oral quercetin (100 mg/kg per day for 4 weeks) on myocardial mitochondrial oxidative stress and dysfunction in adult male BALB/C mice subjected to heavy exercise, probably through its antioxidative effect and aconitase activation, highlighting a promising strategy for EIMD by this naturally occurring flavonoid. Sun et al. [91] reported beneficial effects of a mitochondrial cocktail of nutrients (α-lipoic acid, acetyl-L-carnitine, biotin, nicotinamide, riboflavin, pyridoxine, creatine, coenzyme Q10, resveratrol and taurine) on mitochondrial health in exhaustively exercised rats. Nutrient supplementation increased the protein expression of mitochondrial complexes I, II and III, mtDNA number and transcription factors involved in mitochondrial biogenesis and fusion in skeletal muscle. Similar results are reported by the same group [92], with a combination of mitochondrial targeting nutrients (α-lipoic acid, creatine, B vitamins, polyphenols) caused amelioration of complex V and a FAD-binding flavoprotein enzyme activities, and enhancement of activities of complex I and IV in liver mitochondria of rats subjected to a 4-week strenuous exercise. These two studies suggest that multicomponent mitochondrial nutrient supplementation can reduce EIMD, although the contribution of each nutrient administered remains unknown. On the other hand, Huang et al. [93] reported no significant impact of L-arginine-rich diet (2 %) on common mtDNA4834 deletions in muscular and hepatic mitochondria of rats after exhaustive exercise. No studies are available for other mitochondria-targeted nutraceuticals in EIMD, including small-molecule antioxidants (e.g. mitoquinone, mitotocopherol, mitoapocynin) and molecular hydrogen, designed to accumulate within mitochondria *in vivo* [94,95]. Therefore, further studies are needed to evaluate the full range of mitochondria-targeted interventions for EIMD, including novel treatment approaches (e.g. ketogenic diet, sirtuins, protopanaxadiol) used in mitochondrial medicine [96].

Another controversial aspect of possible antioxidants use in the management of EIMD should be addressed as well. A growing body of evidence suggests rather detrimental effects of antioxidant supplementation during exercise training, with high-dosage antioxidants could adversely interfere with important ROS-mediated physiological processes, such as protein signalling, mitochondrial biogenesis or vasodilation [97–99]. Negative outcomes of antioxidant supplementation were found in cyclists, triathletes, marathon runners, kayakers and non-trained humans supplemented with different antioxidants, both water and lipid soluble [100]. Since the potential for long-term harm of antioxidant supplementation does exist [101], the casual use of high doses of antioxidants in EIMD should perhaps be curtailed until evidence-based guidelines are developed.

CONCLUSION

Mitochondria can efficiently protect themselves from the accumulation of external and internal stress through various quality mechanisms [54,102]. However, when protection mechanisms are tired out or altered due to repetitive exhaustive exercise and inadequate recovery after exercise, EIMD might appear. Although no study followed mitochondrial health (and post-exercise recovery) in a long-term fashion after a single session of exhaustive exercise, it is highly unlikely that a single exercise bout leads to irreparable mitochondrial disturbances, at least in exercise-naïve subjects. However, frequent sessions of exhaustive exercise perhaps do not allow mitochondria to fully recover from exercise stress, and repair severe DNA deletions and ultrastructural damage, as main markers of EIMD. Hypothetically, exhaustive exercise might jeopardize regular mitochondrial life cycle that consists of approximately 5 fusion–fission cycles per hour in a single mitochondrion [103], leading to long-lasting poor mitochondrial performance and health consequences. The literature overview identified possible relationship between exhaustive exercise and mitochondrial dysfunction in humans; however, the findings were limited to cross-sectional studies with no longitudinal cause-n-rule effect studies, confounded by the definition of exhaustive exercise. *In vivo* exercise studies describing 'magnitude threshold' that must be exceeded to irreversibly damage the organelle are warranted, evaluating both clinical and athletic population.

CLINICAL PERSPECTIVES

Extremely heavy or exhaustive exercise fosters mitochondrial disturbances that could permanently damage its function in health and disease. Exercise-induced mitochondrial dysfunction might be a key proxy for heart abnormalities, chronic fatigue and overtraining syndrome, or muscle degeneration in athletic environment. Supporting mitochondrial bioenergetics and helping mitochondrial DNA to repair after exhaustive exercise, and maintaining an optimal antioxidant capacity to scavenge toxic reactive oxygen species inside the organelle comprise possible treatment options for exercise-induced mitochondrial dysfunction.

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