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Name of Journal: *World Journal of Orthopedics*

ESPS Manuscript NO: 27038

Manuscript Type: REVIEW

Dear Editor-in-Chief

World Journal of Orthopedic

We would like to thank you and to thank the reviewers for their time and thoughtful comments. We have made significant changes throughout the text and we feel that we have addressed all of the reviewer comments.

We have changed the wording as suggested by Reviewer 1 to reflect an emphasis on skeletal muscle mitochondrial function in the title, abstract (lines 72, 75, and 78), core tip (lines 87 and 89), and text (lines 123, 388, 411 and 416).

Lines 116 and 118-119 have been changed to reflect the association between mitochondrial dysfunction and disease. References have been added to the first two paragraphs of section 3.2 as requested. Lines 249-257 have been removed. References have been added to lines 281 and 283. Lines 378-387 have been omitted.

We have added a paragraph on the metabolic changes after SCI to section 4. A section on CNS mitochondrial health after SCI including a discussion on respiration of spinal cord tissue has been added (Section 5.1). A discussion of pharmacological agents aimed at increasing mitochondrial biogenesis, many of which are naturally occurring or FDA approved, has been added to the significance and future directions (section 6). This highlights translation to a clinical setting as suggested by reviewer 1's last comment and reviewer 2. A paragraph on blood cells as potential biomarkers of systemic mitochondrial function has also been added in order to address clinical translation as well.

Respectfully,

Ashraf S. Gorgey, MPT, PhD, FACSM

Reviewer 1 comments

1. "The current editorial will review ways to study mitochondrial function (in what tissue type?) and the importance of improving mitochondrial health (in what tissue type?) in clinical populations with a special focus on SCI (in what context?)."
    a. *Page 3 line 77-79: The following sentence was modified to reflect your point: "The current editorial will review ways to study mitochondrial function and the importance of improving **skeletal muscle** mitochondrial health in clinical populations with a special focus on **chronic** SCI."*

2. "How mitochondrial function (in what tissue type?) is impacted after human SCI has yet to be determined. The current review will discuss the importance of studying mitochondrial function (in what tissue type?) after SCI and how these findings could translate to a number of other diseases. In fact, other conditions cited in the review are not necessarily associated with skeletal mitochondrial pathophysiology. So again, the authors must specify and/or tone down their broadly cast description and contentions of "overall mitochondrial health."
    a. *Page 4 line 89-92: We have altered the sentence for clarity. "How **skeletal muscle** mitochondrial function is impacted after human SCI has yet to be determined. The current review will discuss the importance of studying **skeletal muscle** mitochondrial function after SCI ~~and how these findings could translate to a number of other diseases~~.*

3. Accordingly, the authors are highly advised to include one or two paragraphs on CNS mitochondrial health in diseased states or following neurotrauma. In fact, the

authors have only cited one paper on PNS regeneration to rationalize how mitochondrial health may be important for SCI.

   a. *We have added section 5.1 CNS mitochondrial health after SCI to page 13-14 lines 339-356.*

4. The following sentences should be modified to indicate associations/correlations of mitochondrial dysfunction with these abnormal conditions since their cause-effect relationships remains uncertain. "It is well established that damage to the central nervous system by traumatic brain injury, spinal cord injury (SCI) and neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis) results in mitochondrial dysfunction [1]. Recent studies have suggested that metabolic disorders including atherosclerosis, hypertension, cancer, insulin resistance, type II diabetes and obesity result in decreased mitochondrial function as well [2-4]."

   a. *Page 5 line 113-119: We have modified the sentence to reflect your point: "It is well established that damage to the central nervous system (CNS) by traumatic brain injury, spinal cord injury (SCI) and neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis)* ***is associated with*** *mitochondrial dysfunction [1]. Recent studies have suggested that metabolic disorders including atherosclerosis, hypertension, cancer, insulin resistance, type II diabetes and obesity* ***is associated with*** *decreased mitochondrial function as well [2-4].*

5. Authors must provide citations for spectrofluorometric measurements of mitochondrial complex enzymatic activities, first two paragraphs, section 3.2.

   a. *We have added citations to page 9 lines 227-235: "Linked activity between complexes can be measured by adding either NADH (for complex I) or succinate (for complex II) and measuring the reduction of cytochrome c by complex III* ***[18, 19]****. Other key enzymes of the citric acid cycle (i.e., citrate synthase) and β-oxidation pathways (i.e., β-hydroxyacyl-CoA dehydrogenase (β-HAD)) can also be measured spectrophotometrically* ***[18, 20].*** *The benefit of this spectrophotometric analysis is that it uses a small amount of tissue, samples can be previously frozen, and spectrophotometers are common lab equipment* ***[19].****"*

6. Rationale for inclusion of lines 200-208 must be provided as this only describes one tissue type, the muscle. Why is this important for this review, notably in this section for enzyme activity?

a. *Thank you for your comment. We have removed this paragraph per your suggestion, page 10 lines 249-257.*

7. Section 3.3 again only describes respirometry of muscle-derived mitochondria, but what about CNS tissues, notably the spinal cord, which is in the title?
    a. *We have added a paragraph to Section 5.1 (page 14 lines 349-356) which discusses mitochondrial respiration and ETC complex activities in the spinal cord based on your suggestion.*

8. The following sentence exemplifies how muscle-centric this review is, "SCI is usually a result of trauma to the spine, resulting in damage to nerve cells that send messages to and from the brain." The CNS does contain neurons that project nerves into the PNS, but within the spinal cord and brain they are termed neurons.
    a. *Page 11 lines 272-275: We have modified the sentence for clarity: "SCI is usually a result of trauma to the spine, resulting in damage to* ~~***nerve***~~ *cells that send messages to and from the brain.* ***Damage can be to the upper motor neurons that project from the brain to the spinal cord or lower motor neurons that project from the spinal cord to the muscles.***

9. Sentences 228-231 and 284-287 require references.
    a. *Page 11 lines 279-283: Thank you for catching this. We have include references "This immobility, combined with hormonal changes and poor dietary habits result in decreased muscle mass and increased adipose deposition* ***[6, 7, 27].*** *These changes put individuals with SCI at a high risk for developing cardiovascular disease, type II diabetes, and obesity* ***[7, 28, 29].***

10. From sentence 221-273 there is no discussion of metabolic changes in relation to mitochondrial dysfunction, which is enigmatic for this section.
    a. *On Page 13 lines 327-336 we have added Section 4.2, metabolism after SCI "In addition to changes in body composition, metabolism is disrupted after SCI. As many as 55% of individuals with SCI have metabolic syndrome, which is characterized by three or more of the following conditions: obesity, high blood pressure, insulin resistance, high triglycerides, and low high-density lipoprotein (HDL) cholesterol levels [44]. Impaired glucose tolerance was observed in 56% of persons with SCI, compared with only 18% of AB controls [45]. Individuals with SCI also have increased low-density lipoprotein (LDL) cholesterol [46, 47]. These conditions worsen with age and put individuals at risk for developing cardiovascular disease and type II diabetes."*

11. There is no basis for sentence 294-296, purely speculative without reference.
    a. *Based on your suggestion we have removed this sentence from page 15 lines 378-380*

12. Sentences 297-303 are misleading, as alluded to in second paragraph of critiques, because not SCI studies are cited, only PNS studies that have no bearing on concluding sentence.
    a. *Thank you for your suggestion. We have removed this paragraph (page 15 lines 381-387).*

13. "Exercise interventions have been shown to increase mitochondrial function (in what tissue types?) and improve insulin sensitivity in obesity, diabetes, and aging [47, 48]."
    a. *Page 15 lines 388: We have modified the sentence for clarity: "Exercise interventions have been shown to increase **skeletal muscle** mitochondrial function and improve insulin sensitivity in obesity, diabetes, and aging [58, 59]."*

14. In Significance and future directions, the authors are asked to precisely indicate in what type of tissues "mitochondrial function is observed in a number of disease and injury states…" (lines 325-327)
    a. *Page 16 line 411: We have modified the sentence "A decrease in **neuron and skeletal muscle** mitochondrial function is observed in a number of disease and injury states…"*

15. Line 351, "There is limited knowledge regarding mitochondrial health (in what tissue types?) following SCI.
    a. *Page 18 line 470: We have modified the sentence "There is limited knowledge regarding **skeletal muscle** mitochondrial health following SCI"*

16. The authors are asked to provide some insight (examples) regarding the importance of, "developing interventions to increase mitochondrial function and improve metabolic outcomes." Pharmacological, training, and in acute versus chronic conditions?
    a. *We have added a paragraph to page 16-17 lines 419-435 to discuss pharmacological interventions to increase mitochondrial biogenesis "Increasing mitochondrial function by pharmacological activation of mitochondrial biogenesis is an active area of research [67]. There are a number of FDA approved medications as well as naturally occurring substances that activate mitochondrial biogenesis. For example,*

resveratrol, which is found in red wine, activates sirtuin 1 (SIRT1) and increases PGC-1a activity and mitochondrial function and was shown to improve insulin resistance in diabetic patients [68, 69]. Small molecules that activate SIRT1 with improved bioavailability and potency have been developed and are currently being tested in humans. FDA approved pharmacological activators of mitochondrial biogenesis include the β2-adrenergic receptor agonist formoterol [70], the anti-diabetic drug metformin [71], the phosphodiesterase inhibitor sildenafil [72], the PPARγ agonist rosiglitazone [73], the mitochondrial permeability transition pore inhibitor cyclosporine A [74], and the angiotensin-converting enzyme inhibitor captropril [75], among others. Although these compounds are thought to exert their effects at least in part by increasing mitochondrial biogenesis, there are currently no specific activators of mitochondrial biogenesis. Future studies need to investigate the safety and efficacy of systemically increasing mitochondrial biogenesis, as well as optimizing dosing in order to maximize the therapeutic benefit."

b. *We have also added 3 sentences to the conclusion (page 18-19 lines 478-483) "Skeletal muscle or blood cell bioenergetics may predict overall mitochondrial health and therefore be a surrogate marker of disease progression and treatment efficacy. Increasing mitochondrial function immediately following SCI may decrease cell death and improve functional outcomes. Improvement in mitochondrial function by exercise or pharmacological interventions in chronic SCI may decrease comorbidities."*

Reviewer 2

The paper is well written. In order to meet the interest of our readers you shoudl explain wich are the possible clinical impacts of mitochondrial physiology monitoring and manipulation and how translation to clinical setting could take part. Best regards

Thank you for your suggestion. To address this we have added 2 paragraphs to the significance and future directions section, page 16-17 lines 419-450 "Increasing mitochondrial function by pharmacological activation of mitochondrial biogenesis is an active area of research [67]. There are a number of FDA approved medications as well as naturally occurring substances that activate mitochondrial biogenesis. For example, resveratrol, which is found in red wine, activates sirtuin 1 (SIRT1) and increases PGC-1a activity and mitochondrial function and was shown to improve insulin resistance in diabetic patients [68, 69]. Small molecules that activate SIRT1 with improved bioavailability and potency have been developed and are currently being tested in humans. FDA approved pharmacological activators of mitochondrial biogenesis include the β2-adrenergic receptor agonist formoterol [70], the anti-diabetic drug metformin [71], the phosphodiesterase inhibitor sildenafil [72], the PPARγ agonist rosiglitazone [73], the

mitochondrial permeability transition pore inhibitor cyclosporine A [74], and the angiotensin-converting enzyme inhibitor captropril [75], among others. Although these compounds are thought to exert their effects at least in part by increasing mitochondrial biogenesis, there are currently no specific activators of mitochondrial biogenesis. Future studies need to investigate the safety and efficacy of systemically increasing mitochondrial biogenesis, as well as optimizing dosing in order to maximize the therapeutic benefit.

In order to study mitochondrial function after disease or injury or to assess the efficacy of mitochondrial targeted therapies, skeletal muscle biopsies could be used because of the inaccessibility of the brain and spinal cord in humans. However, recent studies have suggested that the bioenergetic profile of blood cells is associated with physical function and inflammation as well [76, 77]. Indeed, mitochondrial dysfunction is seen in blood from patients with a number of diseases including neurodegenerative diseases and type II diabetes [78, 79]. Peripheral blood mononuclear cells from patients with type II diabetes and chronic kidney disease have increased inflammatory cytokines, decreased mitochondrial function and increased ROS production [80]. These studies suggest that blood cell bioenergetics may predict systemic mitochondrial function and may act as biomarkers for metabolic stress and surrogate markers for the severity of disease progression and the efficacy of therapeutics [80, 81]. This represents an intriguing possibility, as obtaining blood samples are much less invasive than biopsies and could be taken more frequently in order to better characterize the time course of therapeutic intervention.

We have also added 3 sentences to the conclusion, page 18-19 lines 478-483 "Skeletal muscle or blood cell bioenergetics may predict overall mitochondrial health and therefore be a surrogate marker of disease progression and treatment efficacy. Increasing mitochondrial function immediately following SCI may decrease cell death and improve functional outcomes. Improvement in mitochondrial function by exercise or pharmacological interventions in chronic SCI may decrease comorbidities."