DOI: https://doi.org/10.53350/pjmhs2115123462

ORIGINAL ARTICLE

The Effect of Aerobic Exercise on Deteriorating VO_2max and Diminished Mitochondrial Biogenesis During Aging

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ABSTRACT

Aging seems to be inevitable and gradual loss of physical activity is associated with frailty and many age-related disorders. Exercise is the way of keeping a healthy life and delaying aging process. Deterioration in pulmonary vital capacity is inevitable, and mitochondrial biogenesis also diminishes with aging. Regular aerobic exercise alleviates the diminishing vital capacity while increasing mitochondrial biogenesis in aging. Peroxisome proliferator-activated receptor c coactivator 1 alpha (PGC-1a), which is the master regulator of mitochondrial biogenesis, is activated by reactive oxygen species (ROS). Exercise-induced lactate leads to formation of ROS and synthesis of nitric oxide (NO) at physiological level. PGC1a regulation by NO seems to be controversial. Over the physiological limit of ROS and NO has toxic effects in cellular environment with reduced antioxidant activities in aging. Overall, exercise seems to be beneficial option to alleviate reduction rate of vital capacity and to enhance mitochondrial biogenesis via lactate-induced ROS formation.

Keywords: Aging, Exercise, Maximum oxygen consumption rate, Lungs vital capacity, Mitochondria Biogenesis.

INTRODUCTION

Aging is associated with insufficiency of cellular and organs' function. Maximum oxygen consumption capacity (VO_2max) diminishes with advancing age. VO_2max is directly affected from the lungs vital capacity and mitochondrial quality and quantity in a cell. The lungs vital capacity irreversibly diminishes during aging. Unfortunately, mitochondrial quality and quantity also deteriorate during aging progress. Furthermore, formation of reactive oxygen species (ROS) and synthesis of nitric oxide (NO) augment with decreasing activities of antioxidant enzymes in aging.

Aging is considered to be in relation to the loss of physical activity (Booth et al. 2012). Exercise is planned and programmed physical activities, which is a required challenge for aging individuals to maintain physical activity at an optimal level. Exercise supports to form structure and functions of a body via an integrated response of an organ system. General types of exercise are aerobic, anaerobic, or combined (Khan et al. 2012). All these types of exercise support being physically active and delaying onsets of age-related diseases. Aerobic type of exercise requires higher rate of oxygen consumption and mitochondrial activity. Aerobic exercise has a special emphasis on vital capacity, mitochondrial quality, and quantity during aging.

Mitochondrial quality and quantity are based on mitochondrial biogenesis, fusion, and fission (Chan 2020; Close et al. 2005). Mitochondrial quality and quantity are primarily determined by mitochondrial biogenesis. Aerobic exercise is reported to enhance mitochondrial biogenesis via lactate induced ROS and NO during exercise. ROS and NO are considered to induce peroxisome proliferator activator receptor coactivator 1 alpha (PGC1a), which is informed to be a master regulator of mitochondrial biogenesis (Close et al. 2005). Therefore, the purpose of study is to evaluate rejuvenation and delaying effects of aerobic exercise on deteriorating VO_2max and reduced mitochondrial biogenesis via PGC1a activation by lactate-induced ROS formation and NO synthesis during aging.

VO2 max decreases in aging due to the reducing lungs vital capacity and restricted mitochondrial biogenesis. In addition, NO synthesis and ROS formation rises above the physiological level in aging. Over-formed ROS and/or NO affects mitochondrial biogenesis both directly and negatively by limiting function of the PGC1a. On the other hand, exercise reduces the declining of vital capacity and maintains the physiological level of ROS during aging. Physiological level of ROS induces PGC1a. PGC1a keeps the endogenic antioxidant at optimum level and works to prevent the reduction of mitochondrial biogenesis (Figure 1). Figure 1 illustrates the effect of being physically inactive or exercising on aging.

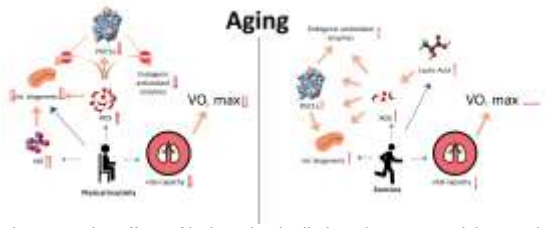

Figure 1. The effect of being physically inactive or exercising on the aging.

mt.biogenesis: mitochondrial biogenesis; ROS: reactive oxygen species; NO: nitric oxide; PGC1a: peroxisome proliferator activator receptor coactivator 1 alpha. ↑: formed at physiological level; ↑↑: over-formed physiological level; ↓: reduced decreasing; ↓↓: decreasing; ↔: protected

VO_2max Capacity During Exercise and Aging: Maximum oxygen consumption capacity decreases with age, which is associated with age-related impairments in pulmonary function. Decreased pulmonary function is a serious limiting factor in the physical activity of elderly. Exercise training does not improve pulmonary function in elderly, which means reduced VO_2max of lungs and lactate threshold (Pollock et al., 2015). The researchers report that VO_2max starts to decline at the age of 30 with a rate of 0.2-0.5 mL/min per kilogram each year (Fleg et. al. 2005). This implies a shift in metabolism from aerobic glycolysis to anaerobic glycolysis.

In elderly pulmonary function is fundamentally based on development of VO_2max capacity in their youthful period (Karlsen et al. 2015). Endurance type of exercise causes to increase aerobic power along with age during childhood in both sexes. Girls attain their VO_2max capacity at about 14 years old, while boys reach their capacity at the age of 18 (Hayes et al. 2013). Exercise is needed for young people to attain their genetic potential and healthy development during growth period (Indranil 2014). Afterwards in advancing ages a decline in VO_2max accelerates from about 0.3 to 0.6% in each year between the ages of 20 to 30, while it accelerates higher than 2% in each year between the ages of 70 to 79 (Fleg et al. 2005). Decrease in VO_2max primarily depends on lungs respiratory capacity and mitochondrial quality and quantity.

Radak et al. (2019) discussed that an improved level VO_2max is related to healthy lungs, heart, muscle, brain, liver and

kidney functions in middle aged and older people. Aerobic metabolic capacity is the maximal oxygen delivery potential to tissues. The higher level of VO_2max is associated with greater muscle oxidative capacity and expression of mitochondrial biogenesis (Trappe et al. 1985). In other words, VO_2max is closely linked to mitochondrial quality and quantity in tissues, which has the primary determinative role in the antioxidant system and formation of ROS.

Free Radical-based Aging, Exercise, and Antioxidant Intake: Among the three hundred plus theories of aging, the free radical theory of aging (Harman, 1956) has attracted much interest and has been studied extensively. This theory proposes that the organism ages as the cumulative effect of oxidative damage. ROS is generated as a by-product of cellular metabolic processes. It has been stated in various studies that ROS shortens the lifespan and at the same time, lifespan is prolonged when ROS is detoxified in some organism (Kirkwood and Kowald 2012). Recently, however, scientists have been skeptical of the free radical-mediated theory of aging. It is not certain that free radicals formation in a life period, shorten the life expectancy, or extend the life expectancy when detoxified. Scientific approaches are now that free radicals have important effects on the quality of life, especially in the elderly, rather than life expectancy. This approach was supported by Deepa et al. (2017). They generated a superoxide-dismutase-knockout mouse (SOD−/-). This animal had high oxidative stress and oxidative damage markers. Interestingly, the animals did not live shorter than the control group of animals. However, frailty started earlier than the controls and the quality of life was declined.

Contractile activity of skeletal muscle is directly related to increased oxygen consumption ratio especially in aerobic exercise. Accordingly, ROS formation elevates in the cellular environment. On the other hand, recently, scientists have been questioning whether mitochondria are radical formation centers in exercise. Mitochondria form more ROS during basal state than active state (maximal ADP stimulated respiration) (Anderson and Neufer 2006). This approach is supported by another study, which states overall percentages of mitochondrial oxygen consumption reveals about 0.15% of superoxide generation (St-Pierre et al. 2002). This amount of superoxide generation is very low to toxify the macro molecules.

Free radicals are generated in metabolism in response to both acute and chronic exercises. Several studies have searched for the effects exercise-formed free radicals on the life expectancy of animals (Deepa et al. 2017; Kirkwood and Kowald 2012; Cao et al. 2012). These studies reveal that physical activity positively affects the quality of life via decreasing age-related physical inactivity, frailty and supporting mental performance in the aged rats but has no effect on the life span. Previously, it was considered that the high rate of free radical formation during exercise might have been deteriorated health. Therefore, the use of large amounts of antioxidants has been recommended, to get rid of the timely effects of free radicals during exercise. However, recent studies revealed contrary findings. It has been demonstrated that high doses of vitamin C in training inhibits exercise adaptation, mitochondrial biogenesis, and synthesis of endogenic antioxidant enzymes (Gomez-Cabrera et al. 2008). It has also been demonstrated that high doses of vitamin C in training inhibits cellular adaptation to exercise, mitochondrial biogenesis, and synthesis of antioxidant enzymes. Later, Ristow et al. (2009) state that antioxidant intake in exercise hindered exercise-related physical performance enhancement and overall health improvement.

Although the effectiveness of free radicals in aging and harmful effects of free radicals in exercise are controversial, nowadays it is considered that moderate level of ROS formation in exercise has a role in cellular signal transduction. Mitochondria is responsible for optimum level of ROS formation during exercise (Power and Jacson 2008). This organelle is at the center of both aging and exercise.

Mitochondria During Exercise and Aging : Mitochondria produce ATP that cells use as a chemical energy or heat to keep body temperature. Mitochondrial quality and quantity retrograde throughout the process of aging (Lyons et al., 2006) and there is energy failure to meet cellular demands (Sahin et al. 2011). Indeed, onset of age-related diseases like Alzheimer's and Parkinson's are reported to have a weakened and reduced mitochondrial function and number (Correia et al. 2016). Mitochondria have a pivotal role in aging due to their importance in cellular oxygen usage and energy production.

Mitochondrial biogenesis occurs in response to various stimuli like cold, exercise, fasting, ROS (Austin and St-Pierre 2012). Exercise augments the mass of mitochondria in the heart, liver, kidney, brain (Marosi et al. 2012) and skeletal muscle (Broskey et al. 2014). Chronic and repetitive type of exercise at low to moderate intensity increases number of mitochondrial genes (Cao et al. 2012). In this study rats swam 10 or 30 minutes in each day for a 5-month period. They also reported that when the exercise lasted 60 or 90 minutes, the number of mitochondrial DNA copies descended. Both exercise duration and period seem to have an impact on mitochondrial quality and quantity. Lifelong exercise leaded to higher mitochondrial quality and quantity in the skeletal muscle of aged individuals over 60 years old. Their aerobic capacity is highly related to the mitochondrial density (Broskey et al. 2014). Indeed, the benefits of regular exercise appear to improve health. Goto et al. (2007) informed that various endogenous antioxidants activities increased by regular repetitive exercise. Regular and lifelong physical activity enhanced and maintained mitochondrial quality and quantity with enhancing age (Zampieri et al. 2015).

Indeed, cellular health can be judged by mitochondrial health. Mitochondria turn into round-shaped from healthier appearance of tubular form with advancing age. This deformity leads to produce more free radicals, which may cause to have a further deformity in shape (Ahmad et al. 2013). Mitochondrial deformities seem to be parallel to reduced VO_2max in aged individuals. Exercise, on the other hand, has potential to lessen a mitochondrial deformity and enhancement of mitochondrial biogenesis in aging. Indeed, aerobic exercise has potential to alleviate a reduction percentage in lungs vital capacity and improve mitochondrial quality and quantity. Therefore, understanding a mitochondrial biogenesis in response to exercise regiments is important for healthy aging.

Mitochondrial Biogenesis via PGC1a: Mitochondrial biogenesis is based on coordination of the nuclear and mitochondrial genomes. There are numerous transcriptional factors in this process. The peroxisome proliferator-activated receptor c coactivator 1 (PGC1) family of transcriptional coactivators seems to have a main role in the regulation of cellular and mitochondrial metabolism. PGC1a, PGC1b and the PGC related coactivator (PRC) are the members of PGC1 family (Handschin and Spiegelman 2006). PGC1a is a master regulator of mitochondrial biogenesis and respiration. Among the identified splice variants of PGC-1a within skeletal muscle, the full-length isoforms (PGC-1a1–3) are related to oxidative phosphorylation and mitochondrial biogenesis (Millay and Olson 2013), while truncated variants like NT-PGC-1a, have a role in muscle hypertrophy (Silvennoinen et al. 2015). Indeed, PGC1a has a significant role in exercise-induced phenotypic changes, substrate utilization and aerobic performance (Handschin and Spiegelman 2006). It also enhances the expression of ROS-detoxifying enzymes i.e., superoxide dismutase 2 (SOD2), and glutathione peroxidase 1 (GPX1) (St-Pierre et al. 2003), which are responsible for detoxification of ROS derivatives (St-Pierre et al. 2006).

PGC-1α co-activates various transcription factors like peroxisome proliferator-activated receptor gamma (PPARgamma), estrogen-related receptors (ERR), nuclear respiratory factors 1 (NRF-1) and 2 (NRF-2), which enhance the expression of nuclear genes being required for mitochondrial biogenesis (Puigserver and Spiegelman 2003). Moreover, mitochondrial gene expression is

controlled by PGC-1α via activation of mitochondrial transcription factor A (TFAM) (Puigserver and Spiegelman 2003). The studies reveal that PGC1a increases mitochondrial biogenesis via regulation of cellular and mitochondrial gene expression, while eliminating toxic effects of ROS through enhancing the expression of ROS-detoxifying enzymes. This balance system may be disrupted by administrating of exogenic antioxidants during exercise, thus may sabotage the exercise adaptation process, mitochondrial biogenesis, and the benefits of exercise.

Regulation of PGC1a During Exercise : During aging the ratio of mitochondrial biogenesis decreases and the exact reason for this is not clear. However, the studies stresses on mitochondrial biogenesis related extra- and intra-cellular regulatory mechanisms. Lactate-induced ROS and NO synthesis in exercise regulates the expression of PGC-1a (Erlich et al. 2016). PGC-1a, activates variety of important genes including genes related to ATP production process and antioxidant enzymes.

PGC1a Regulation by Lactic Acid : Lactate is the end-product of anaerobic glycolysis, which is protonated form of lactic acid. Oxygen availability at rest, leads to transform it into pyruvate. However, anaerobic glycolysis may be an ongoing process even in the resting individuals. Furthermore, aged people have diminished aerobic metabolism due to inevitable decreasing in vital capacity. Exercise-mediated lactate production is considered as means of cellular adaptation. Lactate metabolism generates ROS, which mediates PGC1a up regulation during very preliminary and enhanced stages of endurance exercise, has a role in signal transduction in cellular environment (Figure 2). Exercise duration, intensity and their ration to lactate-induced ROS formation is given in figure 2.

The lactate threshold is an important performance indicator in exercise. In parallel with the increase in exercise load, there is an increase in blood lactate level. Similarly, studies have found an increase in PGC1a consistent with the increase in lactate. Nordsborg et al. (2010) reported an up regulation of PGC1a expression along with blood lactate concentration after bouts of 4 min of cycling exercise performed at 85% of VO2max, comparing with the same procedures of exercise 70% of VO2max. This finding is supported by the other studies. Augmented expression of PGC1a is observed in moderate intensity-loaded exercise than the high-intensity loaded exercise, in the skeletal muscle of a mouse (Brandt et al. 2017). Furthermore, after 3 hours of sodium lactate injection into mouse gastrocnemius muscle, PGC1a expression increased (Kitaoka et al. 2016). PGC1a expression is increased in line with augmented ROS activity, after addition of 20mM sodium lactate into cultured L6 myoblasts (Hashimoto et al. 2007).

Figure 2 demonstrates the relationship between exercise intensity and duration in respect of lactate-induced ROS formation. Physiological level of ROS is maintained at low intensity exercise, which induces PGC1a activation. Further antioxidants enzymes activation and mitochondrial biogenesis take place. However, duration of exercise extends lactate accumulates, which leads to over-formation of ROS. Over-formed ROS surpass antioxidant capacity and prohibits mitochondrial biogenesis (Figure 2).

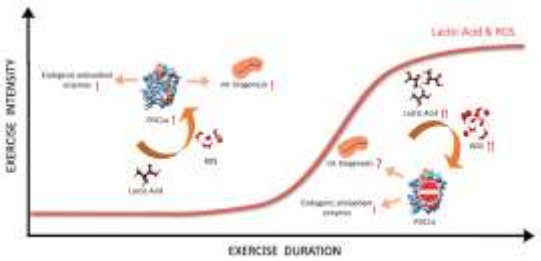

Figure 2. The relationship between exercise intensity and exercise duration.

mt. biogenesis: mitochondrial biogenesis; ROS: reactive oxygen species; PGC1a: Peroxisome proliferator activator receptor coactivator 1 alpha. ↑: physiological level; ↑↑: over-formed physiological level; ↓: decreased.

These studies show that lactate might possibly play a role in cellular adaptation to exercise via ROS formation ending up PGC1a expression. In addition, mild level of lactate might regulate PGC1a expression better than high level of lactate. Since higher lactate level might lead to generation of higher ROS. Resting lactate level might be higher in aged people than young people. Indeed, Muhammad and Allam (2018) reported that aged mice had higher resting blood lactate level than young ones with lower antioxidant enzymes in the gastrocnemius muscle. In this study, when they applied endurance exercise and anti-aging resveratrol supplement separately, they found lower blood lactate level with higher PGC1a and antioxidant enzymes expression in the skeletal muscle. When they supplied resveratrol antioxidant during exercise, they got even better results. This study emphasizes the importance of exercise and antioxidant supplementation in elder individuals, which might lead to lower lactate level with higher PGC1a expression during exercise. Lower lactate level seems to have a role in mitochondrial biogenesis in aged individuals via ROS induced PGC1a expression.

Nitric Oxide Synthesizes in Aging and Exercise: Nitric oxide (NO), which is a diffusible gas, is synthesized in number of cell types via NO synthase's enzymes (endothelial (eNOS), neural (nNOS), and inducible (iNOS) isoforms). NO, which reacts with superoxide to generate peroxynitrite is a strong oxidizing agent. Albeit NO has a critical role in variety of organ systems. Endothelial dysfunction is a common feature in arterial hypertension, atherosclerosis, which are all related to diminish NO bioavailability in aging. In other words, eNOS expression and activity diminishes in aged vascular system. Elevated NO synthesis in aged individuals, which is related to higher iNOS activity, result in higher vasoconstriction tonus in arteries of elder people (Novella et al. 2011).

Navarro and Boveris (2007) inform the altered cytosolic diffusion of NO in aged animals, which is in relation with reduced mitochondrial biogenesis. Exercise induces activity of all three NOS enzymes (endothelial, neural and inducible isoforms) and generation of NO. Indeed, NO synthesis elevates during muscle contraction, while muscle cells generate low ratio of NO at rest (Pye et al. 2007). The effect of aerobic exercise on NOS enzymes were investigated in the soleus and gastrocnemius muscles of aged and young rats (Song et al. 2009). In aged sedentary, iNOS protein and activity level was determined to be high, while the protein level of nNOS is found lower at both skeletal muscles. Besides, the protein level of eNOS was only lower at the white gastrocnemius. After aerobic exercise regimen, both fast and slow twitch muscles had higher protein level of nNOS. In addition, fast twitch muscles only had higher protein level of eNOS. However, eNOS and nNOS enzyme activities were increased in the white gastrocnemius muscle. Exercise did not lead to any significant difference in the protein level and enzyme activity of iNOS in both muscles (Song et al. 2009). These findings show that different mechanisms may be more effective in slow-twitch muscle during aging.

PGC1a regulation via NO in exercise: Albeit mitochondrial biogenesis in skeletal myotubes is induced by NO through up regulation of PGC-1α (Scarpulla 2008), there is controversy about NO regulation of mitochondria. Boushel et al. (2012) informed that NO competes with oxygen in order to bind to a3-heme-site of cytochrome oxidase in low physiological level, and reversibly decreases oxygen consumption of mitochondria. However, NO forms peroxinitrite in higher ratio, which irreversibly inhibits complex I and II of the electron transport chain. On the other hand, up regulation of slow twitch myosin heavy chain (I MHC) during over-load exercise, necessitates NO synthesis (Sellman et al. 2006) by activation of Akt and glycogen synthase kinase-3(GSK-3) and CnA/NFAT-dependent signaling (Drenning et al. 2008). PGC1a regulation by NO seems to be blunted during exercise in

aged individuals. Future research is required in order to clarify regulatory role of NO on PGC1a during mitochondrial biogenesis.

CONCLUSION

Aging is a multidimensional process. One of these dimensions is decreased VO_2max. This reduction is fundamentally due to the decrease in lungs vital capacity in aging. Lungs vital capacity has potential to reach their maximum genetic limits only with exercising in a childhood period. Vital capacity begins to decrease irreversibility with advancing age in twenties. Moreover, diminished mitochondria quality and quantity are the other most important indicators of aging. Regular exercise can reduce the rate of vital capacity declining and at the same time it increases the quality and quantity of mitochondria in advancing ages. Lactate induced ROS has a role in mitochondrial biogenesis during exercise. On the other hand, the effect of exercise-induced NO on mitochondrial biogenesis is blunted. Furthermore, excessive-formed ROS and NO have toxic effects on the cellular compartments. Therefore, high intensity types of exercise might have further toxic effects on aged cellular environment. On the other hand, contrary to common understanding, antioxidant supplementation seems to interfere the beneficial effects of exercise especially in young people. Despite to this, it might be beneficial to the aged people.

In brief, physical activity and exercise seem to be inevitable parts of healthy aging and longevity. Exercising in all age periods seems to be an investment for healthy aging. However, the effect of childhood, middle-aged and/or aged period exercise on healthy aging and longevity necessitates future studies in respect of lungs vital capacities and mitochondrial quality and quantity. In addition, the benefits of antioxidant intake in aged young individuals requires more investigation. Furthermore, exercise schedule for aged/aging individuals should include not only aerobic type of exercise but also it includes other parts of exercise like strength, balance, coordination, and flexibility. One should exercise regularly but stay away from overloading exercise in order to keep free radicals' formation at physiological level and enable mitochondrial biogenesis.

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