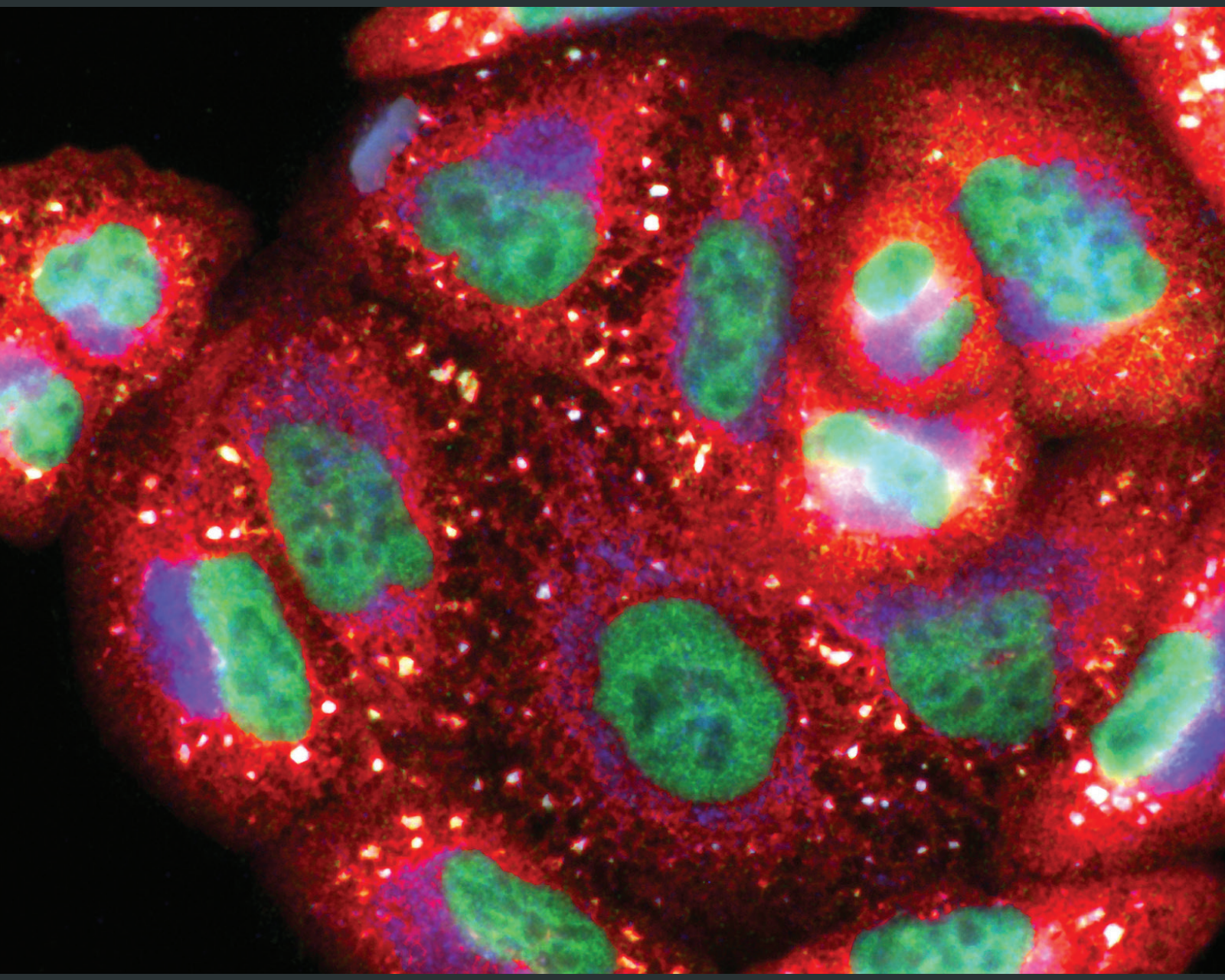
Oxidative Medicine and Cellular Longevity

Exercise, Free Radical Metabolism, and Aging: Cellular and Molecular Processes

Guest Editors: Geraint D. Florida-James, Rickie Simpson, Gareth Davison, and Graeme Close



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Editorial

Exercise, Free Radical Metabolism, and Aging: Cellular and Molecular Processes

Geraint D. Florida-James,[1] Rickie Simpson,[2] Gareth Davison,[3] and Graeme Close[4]

[1]*School of Life Sport and Social Sciences, Edinburgh Napier University, Edinburgh EH11 4BN, UK*
[2]*Laboratory of Integrated Physiology, University of Houston, 3855 Holman Street, Room 104 Garrison, Houston, TX 77204-6015, USA*
[3]*Sport and Exercise Science Research Institute, Ulster University, Jordanstown, Newtownabbey BT37 OQB, UK*
[4]*School of Sport and Exercise Sciences, Liverpool John Moores, Tom Reilly Building, Byrom Street, Liverpool L3 3AF, UK*

Correspondence should be addressed to Geraint D. Florida-James; g.florida-james@napier.ac.uk

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The human aging process is associated with a gradual and cumulative decline in the normal functioning of all major bodily systems. While life expectancy is increasing at an exponential rate, the length of time spent in good health across the lifespan (health-span) is in decline. Exercise and physical activity are widely regarded as important interventions to increase longevity and promote healthy aging and well-being. However, cell damage produced by acute and unaccustomed exercise, as a result of an enhanced production of free radical species, is a recognised phenomenon. While all physiological systems appear responsive to the beneficial effects of exercise, further research is required to understand the relationship between free radical metabolism in cellular and molecular processes affected by exercise in the context of human aging. This special issue contains both review and original research articles which, when combined together, provide an interesting insight into the current state of basic and applied research in free radical metabolism, exercise, and aging.

In their review, O. F. Araneda et al. discuss how environmental factors, such as altitude and pollution, as well as different forms of exercise, intensity, and duration, affect pulmonary inflammation and oxidative imbalance. They review literature pertaining to antioxidants as well as prooxidants, in relation to oxidative damage, and they further discuss mediators of the inflammatory response to exercise. Importantly, they investigate the relationship between oxidative/inflammatory variables and the interaction between them.

D. J. Flis et al. used a prolonged swimming model in rats to investigate mitochondrial swelling and changes in cholesterol in skeletal muscle and liver. A reduction in mitochondrial cholesterol following exercise is related to the inhibition of mitochondrial swelling, and it was also observed within this study that there was an increase in caveolin-1 concentration in muscle. Although markers of oxidative stress in mitochondria were enhanced, there were no similar changes apparent in liver. The work points to a possible adaptive role of caveolin-1 in rat muscle mitochondria, as a consequence of exercise stress.

In the second review of this issue, M. Ross et al. discuss the increased risk of developing cardiovascular disease concurrent with increasing age, with respect to increased and prolonged exposure to oxidative stress. The positive and protective effect of regular exercise, in promoting endothelial homeostasis, which may offset the "vascular ageing" process, is further reviewed.

It is suggested that acute strenuous exercise causes changes to the oxidant/antioxidant ratio and tissue inflammation and, in their contribution to this issue, H. Li et al. add to the literature in this area with their report on the acute effect of exercise and the inflammatory response in the myocardium of rats. The rats were exercised using a treadmill running protocol, and they observed a regulatory adaptation of mitochondrial function in rat myocardium in response to acute heavy exercise. Their work further demonstrates that myocardial injury in rats is minimized via a cascade activation of mitophagy, responding to myocardial

inflammation, as a result of mitochondrial stress triggered during acute exercise.

In the final research article of the issue, A. B. Bigley et al. focus on age, with regard to the effect of latent cytomegalovirus on natural killer cell phenotype and exercise responsiveness in the human model. In response to an acute bout of exercise, they show an increased mobilization of NK-cells, which is thought to be an important component of the "fight or flight" response. They also observed that this response is mediated by age, in reporting an increased proportion of NK-cells expressing the terminal differentiation marker CD57 with advancing years. Additionally, NK-cell mediated immunosurveillance following exercise was reported to be compromised by CMC serostatus irrespective of age.

Although there is certainly great diversity in the papers presented in this special issue, we hope the reader will find the contributions both interesting and stimulating. We suggest that this collection of papers will serve to enrich our understanding and knowledge of the relationship between free radical metabolism in cellular and molecular processes affected by exercise in the context of aging.

Geraint D. Florida-James
Rickie Simpson
Gareth Davison
Graeme Close

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Review Article

Update on the Mechanisms of Pulmonary Inflammation and Oxidative Imbalance Induced by Exercise

O. F. Araneda,[1] T. Carbonell,[2] and M. Tuesta[2,3]

[1]*Laboratorio Integrativo de Biomecánica y Fisiología del Esfuerzo (LIBFE), Escuela de Kinesiología, Facultad de Medicina, Universidad de los Andes, Monseñor Álvaro del Portillo 12455, Las Condes, 7620001 Santiago, Chile*
[2]*Facultad de Biología, Laboratorio de Fisiología e Inmunología, Universidad de Barcelona, Avenida Diagonal 643, 08028 Barcelona, Spain*
[3]*UDA Ciencias de la Salud, Facultad de Medicina, Pontificia Universidad Católica de Chile, Avenida Vicuña Mackenna 4860, Macul, 7820436 Santiago, Chile*

Correspondence should be addressed to O. F. Araneda; ofaraneda@miuandes.cl

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The mechanisms involved in the generation of oxidative damage and lung inflammation induced by physical exercise are described. Changes in lung function induced by exercise involve cooling of the airways, fluid evaporation of the epithelial surface, increased contact with polluting substances, and activation of the local and systemic inflammatory response. The present work includes evidence obtained from the different types of exercise in terms of duration and intensity, the effect of both acute performance and chronic performance, and the influence of special conditions such as cold weather, high altitude, and polluted environments. Levels of prooxidants, antioxidants, oxidative damage to biomolecules, and cellularity, as well as levels of soluble mediators of the inflammatory response and its effects on tissues, are described in samples of lung origin. These samples include tissue homogenates, induced sputum, bronchoalveolar lavage fluid, biopsies, and exhaled breath condensate obtained in experimental protocols conducted on animal and human models. Finally, the need to simultaneously explore the oxidative/inflammatory parameters to establish the interrelation between them is highlighted.

1. Introduction

When doing physical exercise, the usual levels of organic performance are exceeded. However, we are designed to execute the exercise, depending on its variety, duration, intensity, and the environmental conditions under which it is done. The physiological and pathological processes will be activated, which can lead to the generation of an oxidative imbalance and the establishment of an inflammatory process [1, 2]. The oxidative damage happens as an additional cost of using oxygen to obtain energy and can occur when there is an increase in the formation of prooxidants and/or when the antioxidant defense decreases, causing an alteration of tissue product functionality of the structural damage to all the cellular components that contain lipids, carbohydrates, proteins, and nucleic acids [3]. Another response mechanism to physical stress is inflammation, which is triggered as a reaction to the mechanical damage of structural components (connective tissue; muscle, tendon, and bone) and nonstructural components (erythrocytes, endothelium, and epithelia) of the body [4–8]. As a result, stress hormones are released, such as cortisol and catecholamines, which activates the immune system, causing a particular response profile based on the release of soluble mediators (cytokines) and arachidonic acid derivatives (prostaglandins and leukotrienes). The latter and the stress hormones will cause changes in the number and activation of leukocytes subpopulations to the point that intense exercise of long duration can induce immune suppression (increasing the susceptibility to infection) [9], in contrast to the exercise of moderate intensity, which boosts the immune response. Both the alteration of the redox system and the inflammatory reaction have multiple

points of interaction that have been previously evidenced [10–12]. The study of inflammatory/oxidative damage at a pulmonary level has been a topic poorly addressed [13–15], particularly in healthy humans and even more so in athletes. Most of the information in this subject arises from pathophysiology of pulmonary diseases, such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease [16–27]. The lung has the crucial role of gas exchange and experiences great modifications of its activity during the exercise. This mobilizes larger volumes of air and modifies the breathing pattern from nasal to oral, increasing contact with a greater amount of pollutants that may be present in the environment. Also, the lung receives a greater amount of blood flow to increase the exchange in places that are well ventilated, which causes changes in the functioning of the vascular parenchyma [28, 29]. However, the anatomo-functional characteristics of the lungs make it very difficult to obtain information of the redox/inflammatory state in the different sectors of this organ. This work brings together the scientific papers that have addressed the phenomenon of altered pulmonary redox/inflammation environment induced by acute or chronic exercise, in a hypoxic environment, cold or contaminated, in both animal and human models, by focusing on the protocols and mechanisms that explain the phenomenon, as well as their potential implication on those who exercise.

2. Effects of Exercise on the Respiratory System and Its Relationship with the Generation of Oxidative/Inflammation Damage

When exercising, the mobilized air flow or pulmonary ventilation increases. This is explained by the increase of the respiratory rate, the tidal volume, and the appearance of bronchodilation. In addition to this, the pulmonary vascular bed will vasodilate to receive a greater blood flow. These changes, taken together, aim to increase gas exchange. Large air flows entering the lung during exercise will cause a modification of the breathing pattern towards one predominantly oral, favoring the evaporation of the fluid covering the pulmonary epithelium and the decrease of temperature of the airways. As a result, the pulmonary passages will cool down and the osmolarity of the epithelium will increase [30]. It should be noted that the cooling of the pulmonary passages as a result of the hyperventilation has been observed at comfortable environment temperature (+20°C) [31]. In this way, McFadden Jr. and Pichurko [31] showed a decrease of the tracheal temperature of 34°C at pulmonary ventilation of 15 L/min and of 31°C at 100 L/min. The cooling of the airway by hyperventilation produced by exercise is homologous to breathing cold air at rest. The latter is probably in the absence of air pollutants, the main irritative/proinflammatory factor of this region of our body. In cold environments, there is a greater amount of reports of respiratory symptoms [32] and chronic changes of epithelium similar to those of patients with chronically inflamed airways (e.g., asthmatics). Some authors observed, in humans, that the product of intense exercise appears to have similar symptoms to those observed in infection of upper airways [33–35]. However, with moderate training these symptoms decreased [36, 37]. It is probable that intense exercise of long duration, such as a marathon, will increase the susceptibility to infection of the airway by depression of the immune function, contrary to the effect caused by exercise of moderate intensity. Another factor involved in the oxidative/proinflammatory process of the airway is the greater contact with toxic particles and microorganisms present in the environment due to hyperventilation by exercise [38–40]. For example, the damaging effect on lung tissue of environmental substances such as chlorine, ozone, nitrogen oxides, particulate matter, and pollen is recognized [14, 41–43]. The entry of these substances by the pulmonary route can potentially generate systemic inflammation [44, 45] and this will affect the lungs. Finally, another factor of the recognized destabilizing effect of the oxidative balance and in favor of pulmonary inflammation is hypoxia [46, 47]. The general framework for the development of functional changes of the lung by exercise, the activation of the redox imbalance, and the inflammatory system are described in Figure 1.

3. Changes in Pulmonary Redox State and Exercise-Induced Inflammation

As mentioned previously, physical exercise induces changes in the redox/inflammatory state of the organism, at both systemic level and the different organs. In this regard, lung is one of the less studied organs in this context. In the following paragraphs, the most relevant results regarding pulmonary oxidative damage and inflammation caused by exercise are summarized. In this review, the work carried out in healthy subjects was privileged. Regarding the special conditions, hypoxia, water contaminants (chlorine), and cold have been included, leaving aside air pollutants, because there are several reviews regarding this subject [48, 49]. The details of the studies included in terms of goals, characteristics of the sample, the protocol used, and the results related to the pulmonary oxidative/inflammation damage by exercise are summarized in Tables 1 and 2 for human and animals, respectively.

4. Pulmonary Redox Balance and Acute Exercise

A direct relationship has also been reported during exercise, between the acute exercise intensity and the volume of exhaled nitric oxide (VNO), namely, volume minute (VE) multiplied by exhaled nitric oxide (eNO), for sedentary healthy [50, 60, 68, 69, 71, 82, 85–87, 90] and trained subjects [75, 89]. During exercise, eNO have been reported to be decreased when increasing VO_2 [59, 75] and VE [75] in sedentary and active subjects [51, 60, 68, 69, 75, 82, 85, 86, 92]. In athletes, unlike Maroun et al. [75], Kippelen et al. [68] showed changes in eNO during exercise. In animal model, while exercising healthy horses, Mills et al. [112] observed a linear increase of the VNO as the oxygen consumption increased. After exercise, nitric oxide concentrations have

Table 1: (a) Human studies on lung oxidative stress and inflammation induced by acute exercise. (b) Human studies on lung oxidative stress and inflammation induced by chronic exercise.

(a)

Author, year	Aim	Sample's characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Adachi et al. 1997 [50]	eNO and VNO in patients with CHF during exercise*	CHF patients and healthy control subjects (C)	Maximal incremental cycloergometer test in CHF patients (10 W/min) and C (25 W/min) until exhaustion	EB	DE: ↑ VNO during exercise peak in C
Agostoni and Bussotti 2003 [51]	Correlation between eNO and lung mechanics during exercise in CHF*	CHF patients and healthy control subjects (C)	25-W constant workload exercise cycle-ergometry test	EB	DE: ↓ eNO during 3rd and 5th minutes of exercise in C
Araneda et al. 2005 [46]	Lung oxidative damage from exercise at a medium altitude*	Highly trained mountain bikers	Three repetitions of cycle-ergometries of 1 min at maximum intensity in 670 and 2160 MASL with breaks of 1 min	EBC and serum	PE: ↑ [MDA] in EBC, with no changes in serum at 2160 MASL
Araneda et al. 2012 [52]	Duration of a long distance exercise on pulmonary oxidative damage	Amateur runners	Urban 10 km (~53 min), 21 km (~101 min), and 42.2 km races (~246 min)	EBC	PE: ↑ $[H_2O_2]$ and ↑ $[NO_2^-]$ in 21 km and 42.2 km races and no changes in [MDA]; there was a tendency to ↓ of pH
Araneda et al. 2014 [53]	Pulmonary oxidative damage in long distance exercise	Healthy active subjects	10 km race in outdoor athletic track (~50 min)	EBC	PE: ↑ $[H_2O_2]$, ↑ $[NO_2^-]_{EBC}/[NO_2^-]_{Plasma}$ with no changes in the [MDA]; there was a tendency to ↑ of pH
Bikov et al. 2010 [54]	Changes in [Cys-LTs] caused by exercise in asthmatic patients	Nonsmoking asthmatic patients (A) and nonsmoking healthy control subjects (C)	Race on treadmill at a speed and slope maintaining 80–90% HR_{max} (220 − age), which was regulated in 2 min and then maintained during 6 min	EBC	PE: with no changes in [Cys-LTs] in C, but ↑ in A
Bikov et al. 2014 [55]	Changes in EBC_{pH} during EIB in asthmatic patients*	Asthmatics, who reported breathlessness following exercise, and healthy control subjects (C)	Exercise challenge test on a treadmill (details were not described by authors)	EBC and EB	PE: no change of pH in EBC in C
Bonsignore et al. 2001 [56]	Endurance exercise on inflammatory cells in AWs and eNO	Amateur runners	Marathon race (~179 min)	IS and EB	PE: ↑ PMN in IS and ↑ eNO in EB
Bonsignore et al. 2003 [57]	Swimming on inflammatory cells and eNO in the AWs	Swimmers (S) and healthy control subjects (C)	Swimming of 5 km only in the swimmers group, an open pool series (~70 min) and other series in the sea (~54 min)	IS and EB	B: >PMN and <MØ in the IS of S versus C PE: ↑ eosinophils, ↑ lymphocytes, and ↓ MØ in the sea versus swimming pool; eNO was > in the sea in comparison to swimming pool

(a) Continued.

Author, year	Aim	Sample's characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Carbonnelle et al. 2008 [58]	eNO after swimming sessions*	Trained healthy young people, not trained with swimming	Swimming in 2 sessions of 45 min (~1300 m), in a disinfected pool with [NaClO] and another sanitized with electrical process	EB	PE: ↑ eNO only in sanitized pool
Chimenti et al. 2009 [40]	Inflammation of the AWs in urban races in different climatic seasons*	Amateur runners	21 km race in autumn (~89.1 min), 12 km race in winter (~46.1 min), and 10 km race in summer (~35.4 min)	IS	B: ↑ PMNs with ↑ [TNF-α] and ↑ [IL-8] PE: PMNs tended to ↑
Chimenti et al. 2010 [5]	Damage and inflammation of the lung epithelium in a long distance exercise	Amateur runners and healthy control subjects	20 km outdoor races (~90 min)	IS and serum	PE: ↑ [IL-8] in IS and ↑ CC16 in serum
Chirpaz-Oddou et al. 1997 [59]	eNO and VNO during exercise	Healthy control and trained subjects	Incremental cycloergometry to exhaustion with 5 min of passive recovery in sedentary subjects (♂ ~30 min and ♀ ~20 min) and trained subjects (~14 min)	EB	DE: ↓ eNO progressive with ↑ exercise intensity from 65% VO_2max and ↑ VNO with the ↑ of the intensity of exercise > 30 W in all subjects
Clini et al. 2000 [60]	To evaluate eNO during exercise in patients with stable COPD*	COPD patients and healthy control subjects (C)	Maximal cycle-ergometry test (cadence: 60 cycles/min and load: 10 W/min) until exhaustion	EB	DE: ↓ eNO at peak exercise and ↑ VNO in C
De Gouw et al. 2001 [61]	Role of eNO in the airway response to exercise by using L-NMMA, L-arginine, or placebo as pretreatment to exercise challenge*	Asthmatic patients and healthy control subjects (C)	Cycle-ergometry for 6 min using dry air, while ventilation was kept constant in 40–50% of his or her predicted maximal voluntary ventilation ($35 \times FEV_1$)	EB	PE: ↑ eNO 30 min after exercise in C
Denguezli-Bouzgarrou et al. 2006 [62]	Endurance exercise and inflammatory cells of the AWs	Long-distance runners	Races on treadmill at 80% of MAS (~60 min)	IS	PE: ↑ PMNs, ↓ MØ, and ↑ lymphocytes
Denguezli-Bouzgarrou et al. 2007 [63]	Inflammatory mediators, cellular composition in AWs, and acute exercise during a sports season	Long-distance runners	Race at 80% MAS during the basic, precompetitive, and competitive period of a sport season in 1 year (~60 min)	IS	PE: ↑ PMNs in the precompetitive and competitive period. ↑ MØ in the precompetitive period; also, ↑ [histamine], ↑ [IL-8], ↑ [LTB_4], and ↑ [LTE_4] in the competitive phase
Evjenth et al. 2013 [64]	To investigate the effect on FE_{NO} of a standardized exercise challenge test on a treadmill*	Nonasthmatic children with and without allergic rhinoconjunctivitis (AR) symptoms	Run on treadmill (6 to 8 min); heart rate target during the last 4 min was 95% of predicted maximum heart rate (220 − age)	EB	PE: ↓ eNO in nonasthmatic children without allergic rhinoconjunctivitis

(a) Continued.

Author, year	Aim	Sample's characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Font-Ribera et al. 2010 [65]	Inflammation and postexercise pulmonary oxidative stress*	Healthy subjects	Swimming in a chlorinated indoor-swimming pool (40 min), whose average speed was 22.5 ± 9.7 m/min	EBC and EB	PE: no changes of eNO in EB; [RANTES], [IL-12p70], [IFN-γ], [IL-4], [IL-8], [IL-10], [IFN-γ-induced protein 10], [TNF], [VEGF], and [8-isoprostane] in the EBC were not modified
García-Río et al. 2006 [66]	FE_{NO} before and after exercise challenge in patients with asthma and its relationship with airway obstruction*	Nonsmoking, steroid-naïve, atopic patients with mild persistent asthma and nonsmoking, nonatopic, healthy subjects (C)	Performing an exercise challenge on a cycloergometer, with monitored ventilation (exercise parameters were not presented)	EB	PE: with no changes in eNO of healthy subjects
Hopkins et al. 1997 [67]	Pulmonary capillary pressure and function of the alveolar-capillary barrier during intense exercise*	Athletes with signs of hemoptysis by exercise and healthy control subjects	4 km cycling with 12% hill sloping during ~7 min	BALF	PE: >alveolar MØ, >[LTB_4], and < lymphocytes in athletes versus control subjects
Kippelen et al. 2002 [68]	eNO level in endurance-trained athletes during and after intense exercise*	Nine athletes with exercise-induced hypoxaemia (EIH), 12 athletes without EIH, and 10 untrained subjects	15 min intense cycling exercise at 90% VO_2max	EB	DE: ↓ eNO and ↑ VNO (last 3 minutes) in all groups
Larsson et al. 1998 [32]	Cold air and inflammation in the AWs during rest and exercise*	Healthy subjects	Race on treadmill at −23°C and +22°C, each with 4 stages with 15 min at moderate intensity and 15 min of recovery	BALF	PE: at −23°C ↑ granulocytes and ↑ MØ; no changes in [IL-8]
Lovell et al. 2000 [69]	eNO and incremental exercise test in chronic congestive cardiac failure*	Chronic congestive cardiac failure patients and healthy control subjects (C)	Performing Bruce protocol modified by inclusion of an initial 3 min stage at 5% incline, later performing a constant workload test (6 min at 2.7 km h^{-1} and 5% incline)	EB	DE: ↓ eNO and ↑ VNO during Bruce test in C; ↑ VNO during constant workload test
Mantione et al. 2007 [70]	eNO breath levels just before engaging in their respective activity	Healthy control subjects	Going up and down the stairs on a 20-foot staircase for 2 min	EB	PE: ↓ eNO 1 minute after exercise
Matsumoto et al. 1994 [71]	eNO and VNO during exercise	Healthy subjects	Cycle-ergometry at 100 W and maximum intensity with 5 min of recovery (~13 min)	EB	DE: ↑ VNO at 100 W and at maximum pedaling intensity
Marek et al. 2008 [72]	[L-lactate] and [H_2O_2] during exercise*	Healthy subjects	Cycle-ergometer steady-state exercise at 60 W (~7 min) and 120 W (~5 min)	EBC	DE: ↑ [L-lactate] and ↑ [H_2O_2] in 60 W and 120 W
Marek et al. 2009 [73]	Maximal exercise, H_2O_2 release rate, and acid-base status	Amateur athletes	Incremental cycloergometry to exhaustion (~13 min)	EBC	PE: ↑ [H_2O_2] with no changes in pH nor [HCO_3^-]

(a) Continued.

Author, year	Aim	Sample's characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Marek et al. 2013 [74]	Exercising in cold weather and release of H_2O_2*	Healthy subjects	Races on treadmill at 75–80% HR_{max} at ~18°C and ~−15°C (~50 min)	EBC	PE: ↑ $[H_2O_2]$ and ↑ rate of H_2O_2 release in both temperatures
Maroun et al. 1995 [75]	Physical condition and release of eNO during exercise	Healthy sedentary subjects (S), active subjects (Ac), and athletes (A)	Cycle-ergometries in steady-state at 1 and 2 L/min of VO_2 only performing an additional one at 4 L/min of VO_2	EB	PE: ↓ eNO at >VO_2 in S and Ac; ↑ lineal of VNO with ↑ VO_2 in A
Mercken et al. 2005 [76]	Exercise-induced oxidative stress in COPD*	COPD patients and healthy control subjects (C)	Incremental cycle-ergometry exercise test until exhaustion and submaximal constant work rate exercise test (60% maximal power output)	EBC	PE: ↑ $[H_2O_2]$ in maximal but not in submaximal exercise in C
Mercken et al. 2009 [77]	Pulmonary oxidative stress by endurance exercise in COPD and healthy subjects*	COPD patients and healthy control subjects	Cycle-ergometry on one leg at 40% of maximum power output (20 min)	EBC	PE: ↑ $[H_2O_2]$ in COPD patients but not in healthy control subjects
Morici et al. 2004 [78]	VE during exercise and inflammation in the AWs	Young rowers	Maximal run of 1000 m on the rower ergometer (~3 min)	IS	DE: ↑ tendency in epithelial cells at a higher VE PE: ↑ MØ with both ↑ VE/kg and ↑ VT/kg
Nowak et al. 2001 [79]	Prooxidants and oxidative damage by moderate exercise	Healthy subjects	Cycle-ergometer exercise test at 120 W during 6 min or until a HR of 120 bpm is reached	EBC	PE: with no changes in $[H_2O_2]$ and [TBARs]
Nadziakiewicz et al. 2006 [80]	Effects of the physical activity on eNO levels in healthy subjects and in CAD patients*	CAD patients and healthy control subjects smokers and nonsmokers	Bruce protocol exercise test	EB	PE: without changes in eNO in healthy control subjects nonsmokers
Pedersen et al. 2009 [81]	Inflammation in the AWs after 1-exercise session	High performance swimmers	Swimming in indoor-swimming pool at moderate intensity (45 min) whose average heart rate was 162 bpm	EBC and IS, EB	PE: no changes in the cellular composition in IS, eNO in EB, nor pH in EBC of swimmers
Pogliaghi et al. 1997 [82]	VNO after modifying pulmonary blood flow with head-out water immersion or increased gravity at rest and during exercise*	Nonsmokers and healthy subjects who underwent air with normal conditions, water immersion, or increased gravity (1 Gz or 2 Gz)	Incremental cycle-ergometry test, loading was increased progressively by 50 W every 3 min until voluntary exhaustion	EB	DE: ↓ eNO and ↑ VNO in all groups
Pucsok et al. 2007 [83]	Lung PGE_2 and TXB_2 and exercise	Judo competitors	Incremental run on treadmill until VO_2max is reached (run time was not recorded)	EBC	PE: ↑ $[PGE_2]$ and ↑ $[TXB_2]$ in ♂, but not in ♀
Riediker and Danuser 2007 [84]	Low-intensity physical activity and pH	Healthy subjects	Walk on treadmill at 60% HR_{max} predicted with 1 min pause every 10 min (~30 min)	EBC	PE: ↑ pH

(a) Continued.

Author, year	Aim	Sample's characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Riley et al. 1997 [85]	NO production in patients with abnormalities of the pulmonary circulation*	PPH (primary pulmonary hypertension), PF (pulmonary fibrosis), and normal subjects group	Maximal (20 W/min in the normal subjects and 15 W/min in the PF patients and individual estimated exercise tolerance in PPH patients) and submaximal constant work rate cycle-ergometry exercise test (work rate VO_2 midway between each patient's anaerobic threshold and VO_2max)	EB	DE: ↓ eNO and ↑ VNO in normal subjects at peak exercise in maximal and constant work rate exercise test
Rolla et al. 2003 [86]	Relationship between eNO and exercise tolerance in patients with moderate MS*	Patients with moderate MS and healthy control subjects (C)	Symptom-limited incremental exercise test with an upright cycle-ergometer (25 W every 3 min until exhaustion)	EB	DE: ↓ eNO and ↑ VNO in all groups at the end of exercise
Shin et al. 2003 [87]	Relationship between exercise and NO exchange	Nonsmoking healthy adults	High-intensity exercise treadmill test at 90% of the predicted maximum heart rate (220 − age in years) for 20 min	EB	PE: ↑ VNO
St Croix et al. 1999 [88]	Effect of exercise on endogenous NO formation by measuring eNO at a constant airflow rate	Healthy, nonasthmatic, and nonsmoking subjects	3 min of constant-load cycle-ergometry exercise test at three different exercise intensities corresponding to 30%, 60%, and 90% VO_2max	EB	PE: ↓ eNO and ↑ VNO for all intensities of exercise in healthy subjects
Therminarias et al. 1998 [89]	Exercise in cold air on eNO and VNO*	Highly trained subjects (cross-country skiers, triathlon, and running)	Incremental cycloergometry to exhaustion in a climate chamber at +22°C and −10°C (~30 min)	EB	DE: ↓ eNO with the ↑ of the intensity >60 W in +22°C and ↑ VNO with the ↑ of the intensity >30 W in both temperatures
Trolin et al. 1994 [90]	eNO and VNO during exercise	Healthy subjects	Moderately heavy exercise on a cycloergometer (♀: 90 W for women and ♂: 150 W for ♂)	EB	DE: ↓ eNO
Tufvesson et al. 2013 [91]	Relationship between CC16 levels in plasma and urine after exercise with exhaled breath temperature and eNO*	Asthmatic and healthy control subjects	During first six minutes speed and slope were adjusted to maintain the heart rate subject to 90% of their theoretical maximum heart rate (220 − age); the next two minutes were adjusted again to reach maximum effort	EB	PE: ↓ eNO in both groups

(a) Continued.

Author, year	Aim	Sample's characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Verges et al. 2006 [92]	Effect of prolonged exercise on the NO concentration in the lung	Nonsmokers undertaking a moderate to intense training program participated in the study	100 min exercise test was performed on a cycle-ergometer (5 min of rest, 30 min warm-up at 25% P_{max}, 10 min at 60% P_{max}, 2 min at 25% P_{max} repeated five times (S1 to S5), and 10 min of active recovery at 25% P_{max})	EB	DE: ↓ eNO for all exercise sessions (WU, S1 to S5, and active recovery)
Wetter et al. 2002 [93]	EIAH and pulmonary inflammation*	Endurance athletes with EIAH who used anti-inflammatory or placebo	Maximal incremental run on treadmill to exhaustion (~18 min)	IS	PE: with no PMNs, lymphocytes, nor MØ; ↑ [Histamine] in placebo
Yasuda et al. 1997 [94]	To examine the origin and role of eNO during exercise	Healthy control subjects	Two sets of 10 minutes in a cycle-ergometer (5 min without load and 5 minutes with 60 W and 60 RPM) separated, with 15 minutes between them	EB	DE: with no changes in eNO
Zietkowski et al. 2010 [95]	To assess the possible association of EIB with low-grade systemic inflammation in asthmatic patients*	Asthmatics (14 with EIB, 10 without EIB) and healthy volunteers	Cycle-ergometer test for 9 min with a fixed workload adjusted to increase the heart rate to 85% of the maximum predicted for the age of each patient	EBC	PE: with no changes in hs-PCR in healthy volunteers

AWs: airways; BALF: bronchoalveolar lavage fluid; CAD: coronary artery disease; CC16: Clara cell secretory protein; CHF: chronic heart failure; COPD: chronic obstructive pulmonary disease; Cys-Lts: cysteinyl leukotrienes; EB: exhaled breath; EBC: exhaled breath condensate; EIAH: exercise-induced arterial hypoxemia; EIB: exercise-induced bronchoconstriction; eNO: exhaled nitric oxide; FE_{NO}: fractional exhaled nitric oxide; HCO_3^-: bicarbonate; H_2O_2: hydrogen peroxide; HRmax: maximum heart rate; IFN-γ: interferon gamma; IFN-γ-induced protein-10: interferon-gamma-induced protein-10; IL-12p70, IL-4, IL-8, and IL-10: interleukin-12p70, interleukin-4, interleukin-8, and interleukin-10; IS: induced sputum; L-NMMA: N-monomethyl-L-arginine; L-lactate: lactate; LTB_4: leukotriene B_4; LTE_4: leukotriene E_4; MØ: macrophages; MAS: maximal aerobic speed; MS: mitral stenosis; MDA: malondialdehyde; MPO: myeloperoxidase; MASL: meters above sea level; NaCLO: sodium hypochlorite; NO_2^-: nitrite; NO output: nitric oxide output (eNO × VE); PGE_2: prostaglandin E_2; P_{max}: maximal power output; RANTES: regulated upon activation, normal T-cell expressed, and secreted; TBARs: thiobarbituric acid reactive species; TNF(-α): tumor necrosis factor (alpha); TXB_2: thromboxane B2; Se: selenium; VE: minute ventilation; VEGF: vascular endothelial growth factor; VNO: volume of nitric oxide; VO_2max: oxygen uptake (maximal); VT: tidal volume. In "*Oxidative or inflammatory main results,*" DE: during exercise and PE: postexercise. In "*Aim,*" *the effect of exercise was not the primary aim of the study.

(b)

Author, year	Aim	Sample's characteristics	Experimental protocols	Samples obtained	Oxidative or inflammatory main results
Belda et al. 2008 [96]	Type of sport (aquatic or terrestrial) and cell count*	Elite healthy athletes and with asthma	Comparison of baseline samples between healthy and asthmatic athletes who practice water sports in pools or terrestrially (T: ~20 h/wk, with the exception of healthy subjects in water with T: ~10 h/wk)	IS	There was a positive correlation between PMNs with training time and water sport in the pool
Carraro et al. 2006 [97]	eNO in regular attendance to swimming pools*	Children swimmers attending and control children not attending the swimming pool	Comparison of baseline samples between swimmers who attended a swimming pool (1 h/week/6 months) and control subjects	EB	There were no differences in eNO between both groups

(b) Continued.

Author, year	Aim	Sample's characteristics	Experimental protocols	Samples obtained	Oxidative or inflammatory main results
Ferdinands et al. 2008 [98]	Exercise in contaminated environment and inflammation	Cross-country athletes and healthy control subjects	Comparison of baseline samples before and after 10 workouts in 15 d (~1 h/d)	EB	<pH in cross-country athletes compared to their control subjects between their respective sample times
Heinicke et al. 2009 [47]	Pulmonary oxidative damage and prolonged stay in medium height training*	Biathletes and sedentary control subjects	Comparison of baseline samples between biathlete (T: ~5 h/wk) and control subjects; both groups were exposed to 2800 MASL during the 6 weeks	EBC	[H_2O_2] and [8-isoprostane $PGF_2\alpha$] with no differences between groups; by gathering data ↑ [H_2O_2] and tendency to ↑ [8-isoprostane $PGF_2\alpha$]
Helenius et al. 1998 [99]	AWs inflammation in swimmers	Elite swimmers and nonathletic control subjects	Comparison of baseline samples between swimmers (T: 800–3380 km/year) and control subjects	IS	>Eosinophils, >PMNs, >[EPO], and >[human neutrophil lipocalin] in swimmers in comparison to control subjects
Helenius et al. 2002 [100]	Retirement from swimming in relation to AWs inflammation	High performance swimmers	Comparison of baseline samples between active (T: ~1870 km/year) and inactive swimmers (3 months of inactivity)	IS	>eosinophils and >lymphocytes in active swimmers than inactive swimmers
Karjalainen et al. 2000 [101]	Inflammatory cells in skiers, mild asthmatics, and healthy control subjects*	Elite healthy skiers and nonathletic control subjects	Comparison of baseline samples between skiers (T: 200–630 h/year) and control subjects	Endobronchial biopsy	>lymphocytes-T (43 times), >MØ (26 times), >eosinophils (2 times), and >PMNs (2 times) in skiers in comparison to control subjects
Martin et al. 2012 [102]	AWs inflammation and exposure to swimming pool in athletes*	Endurance athletes	Comparison of baseline samples of pool based (5 h/wk) and non-pool-based (0.5 h/wk) athletes (T: ~15 h/wk)	EB and IS	PMNs and eosinophils in IS and eNO in EB were not different between groups
Sue-Chu et al. 1999 [103]	AWs inflammation in skiers	Cross-country skiers and nonathletic control subjects	Comparison of baseline samples during the competitive period, in autumn and winter, between skiers (T: 435 h/year) and control subjects	BALF	>total cells, >lymphocytes, and >mast cells in skiers in comparison to control subjects, with no differences in [TNF-α] and [MPO]
Sue-Chu et al. 2000 [104]	Budesonide and AWs inflammation in skiers*	Elite cross-country skiers with asthmatic symptoms and budesonide or placebo supplementation	Comparison of baseline samples among skiers, after 20 weeks of supplementation with 800 μg/d budesonide (T: ~427 h/year) or placebo (T: ~468 h/year)	BALF and endobronchial biopsy	Lymphocytes, MØ, eosinophils, PMNs, and mast cells were not different between groups

AWs: airways; BALF: bronchoalveolar lavage fluid; EB: exhaled breath; EBC: exhaled breath condensate; EPO: eosinophil peroxidase; H_2O_2: hydrogen peroxide; IS: induced sputum; 8-isoprostane PGF2α: 8-isoprostane prostaglandin F_2 alpha; MØ: macrophages; MPO: myeloperoxidase; NO: nitric oxide; PMNs: polymorphonuclear neutrophils; T: training volume; TNF-α: tumor necrosis factor-alpha. In "*Aim*," *the effect of exercise was not the primary aim of the study.

Table 2: (a) Animal studies on lung oxidative stress and inflammation induced by acute exercise. (b) Animal studies on lung oxidative stress and inflammation induced by chronic exercise.

(a)

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Akil et al. 2015 [105]	Se administration affects lipid peroxidation in liver and lung tissues of rats subjected to acute swimming exercise*	Sprague-Dawley adult male rats divide into general control, Se-administered, swimming control, and Se-administered swimming groups	Swimming was performed once for 30 minutes	Lung tissue	PE: ↑ MDA and ↑ GSH in swimming control versus general control
Al-Hashem 2012 [106]	VitE and VitC in protection of pulmonary damage induced by exercise in altitude*	Wistar rats with 6 months of altitude adaptation	Forced swimming for 2.5 h in glass tank at 600 and 2270 MASL in accordance with altitude adaptation	Lung tissue	PE: ↑ [TBARs], ↓ SOD, and CAT activity at 600 MASL Supplementation with VitE and VitC reversed these results
Caillaud et al. 1999 [107]	Effect of acute exercise on lipid peroxidation in lung compared with locomotor muscles*	Wistar rats exercised (E) and control rats (C)	Race on treadmill at 28 m/min and 15% grade (80–85 VO_2max) until exhaustion (~66 min)	Lung tissue	PE: no changes of pulmonary activity of SOD, CAT, and [MDA] of E in comparison to C
Cathcart et al. 2013 [108]	Effects of exercise during different ambient temperatures and humidity on eNO, eCO, and pH	Thoroughbred racehorses	Exercised under saddle on an all-weather 1.6 km track at half-pace canter, full-pace canter, or gallop according to the current training regimen for each horse	EBC and EB	PE: only ↑ pH in EBC
Hatao et al. 2006 [109]	Acute exercise and antioxidant enzyme activation in aged rats*	Young rats (YR) or aged rats (AR) exercised (E) or not exercised control (C)	Race on treadmill at 25 m/min for YRE and 18–20 m/min for ARE for 60 min	Lung tissue	PE: ↑ Mn-SOD activity in YRE and ARE in comparison to their control subjects; ↑ CuZn-SOD and CAT activity in YRE and ↓ reactive carbonyls derivative in ARE, in comparison to their control subjects
Huang et al. 2008 [110]	Supplementation with L-Arg on pulmonary inflammation and oxidative damage induced by exercise in aged rats*	Sprague-Dawley rats exercised (E) or sedentary (S) with L-Arg (+L-Arg) or without control rats L-Arg (C)	Race on treadmill for groups E at ~70% VO_2max until exhaustion (time for E+L-Arg and EC ~63 and ~51 min, resp.)	Lung tissue	PE: ↑ [XO], ↑ [MPO], and ↑ [MDA] in EC in comparison to SC; with no changes between EC and SC for [SOD], [CAT], [GSH-Px], [GR], and [GSH]
Kirschvink et al. 2002 [13]	Oxidative state, pulmonary function, and airway inflammation in healthy horses and with arcades*	Trained healthy horses, affected by arcades or clinical remission	Race on treadmill with 2 min to 8, 9, and 10 m/s and 4% inclination, stages interrupted by 2 jogs of 8 min to 3.5 m/s (10 min of warming up and 10 min of recovery)	BALF	PE: ↑ [UA] in healthy horses

(a) Continued.

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Lin et al. 2005 [111]	Oxidative stress and antioxidant defenses in animals supplemented or not with L-Arg*	Sprague-Dawley rats grouped as exercised (E) or sedentary (S) with L-Arg (+L-Arg) or control rats without L-Arg (C)	Race on treadmill for E groups at 20 m/min for 15 min and 25 m/min for 30 min; then they run at 30 m/min and 10% of inclination (70–75% VO_2max) until exhaustion (EC ~81 min and E+L-Arg ~87 min)	Lung tissue	PE: ↑ activity XO and MPO in EC in comparison to SC; ↑ [UA], ↑ [NO], and ↑ [MDA] in EC in comparison to SC; ↑ activity SOD and GR in EC in comparison to SC
Mills et al. 1996 [112]	eNO and VNO during acute exercise	Healthy horses	Maximal incremental race until 9 m/s	EB	DE: positive correlation of eNO and VNO with the race intensity
Radák et al. 1998 [113]	Acute anaerobic exercise and oxidative modification of pulmonary proteins	Exercised Wistar rats (E) and sedentary control rats (C)	Two races on treadmills at 30 m/min for 5 min; after 5 min of recovery, a 3rd race to exhaustion was performed	Lung tissue	PE: >pulmonary carbonyls and [glutamine synthetase] in E versus C
Reddy et al. 1998 [114]	Pulmonary oxidative damage by acute strenuous exercise in rats deficient in Se and VitE	Female Wistar albino rats deficient in Se and VitE and control rats	Intense swimming to exhaustion	Lung tissue	PE: >[SOD] and <[GSH-Px] and <[GST] in rats deficient in VitE and in comparison to control rats
Prigol et al. 2009 [115]	Supplementation with $(PhSe)_2$ and pulmonary oxidative damage caused by the exercise	Adult Swiss albino mice supplemented with $(PhSe)_2$ and not supplemented control mice	Swimming exercise (20 min) for both groups after 7 d of supplementation	Lung tissue	PE: ↑ [MDA] and ↑ of CAT activity in mice not supplemented with $(PhSe)_2$
Terblanche 1999 [116]	Exhaustive swimming and CAT activity in the lungs of male and female rats*	Sprague-Dawley rats	1 h swimming	Lung tissue	PE: ↑ CAT activity in males and females

BALF: bronchoalveolar lavage fluid; CAT: catalase; $(PhSe)_2$: diphenyl diselenide; GR: glutathione reductase; GSH: glutathione reduced; GSH-Px: glutathione peroxidase; GST: glutathione S-transferase; L-Arg: L-arginine; MASL: meters above sea level; MDA: malondialdehyde; MPO: myeloperoxidase; NO: nitric oxide; Se: selenium; SOD: superoxide dismutase; CuZn-SOD: copper-zinc-superoxide dismutase; Mn-SOD: manganese-superoxide dismutase; TBARs: thiobarbituric acid reactive substances; UA: uric acid; VNO: volume of nitric oxide; XO: xanthine oxidase; VitE: vitamin E; VitC: vitamin C. In "*Oxidative or inflammatory main results,*" DE: during exercise and PE: postexercise. In "*Aim,*" *the effect of exercise was not the primary aim of study.

(b)

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Altan et al. 2009 [117]	SOD activity and [TBARs] postadaptation by training in altitude*	Wistar albino rats divided into trained in hypobaria (THb) and normobaria (TNb) and nontrained in hypobaria (Hb) and normobaria (Nb)	Comparison of baseline samples between groups trained with swimming (T: 5 at 30 min/day/for 4 days/week for 9 weeks) or nontrained and exposed or not to simulated altitude of 3000 MASL (E: 120 min/day for 4 days/week for 9 weeks)	Lung tissue	PT: >SOD activity in TNb in comparison to Nb; no differences in [TBARS] for the same groups

(b) Continued.

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Asami et al. 1998 [118]	DNA oxidative damage by chronic exercise	Sprague-Dawley rats with spontaneous (S), forced (F) exercise and sedentary control rats (C)	Comparison of baseline samples among rats with spontaneous exercise (wheel), trained on treadmill (T: 30–90 min/day for 25 days), and control rats	Lung tissue	PT: >[8-OH-dG] in F in comparison to S; the DNA oxidative damage was related to the exercise intensity
Aydin et al. 2009 [119]	Long period of dietary restriction and stress produced by high intensity swimming*	Sprague-Dawley rats with restricted diet (RD) or ad libitum (AL), grouped in trained (+T), exercised (+E), and sedentary control rats (C)	Comparison of baseline samples of RD and AL in +T (T: 8 weeks of swimming with 2% BW as extra load during ~50–80 min), PE in +E (E: swimming until exhaustion), and baseline C	Lung tissue	PT: <GSH activity and >GSH-Px of AL+T compared to ALC; <LPO, >GSH, and GSH-Px in AL+E that AL+T PE: ↑ [MDA], ↓ [GSH], ↓ GR activity, and ↑ GSH-Px of AL+E compared to ALC (acute effects)
Chimenti et al. 2007 [120]	Epithelial remodeling, inflammatory cells, and apoptosis in the AWs after chronic exercise	Trained Swiss mice (T) and sedentary control mice (C)	Comparison of baseline samples among trained mice (T: 5 d/week for 6 wk at moderate to high intensity)	Lung tissue	PT: >apoptosis, >proliferation, >loss of hair cells, and infiltration of leukocytes in the AWs in T versus C
da Cunha et al. 2013 [121]	Chronic exercise on oxidative stress and NF-κβ/p65 pulmonary immunocontent of rats with lung injury	Trained Wistar rats (T) and nontrained control rats (C)	Comparison of baseline samples among rats trained on treadmill (T: 20 min at 60% VO_2max during 24 days in 3 months)	BALF and lung tissue	PT: >pulmonary catalase activity in T versus C; there are no changes in [TBARs], carbonyls, dichlorofluorescein, [NO_2^-], and NF-κβ/p65 in the lung
Gündüz et al. 2004 [122]	Oxidant and antioxidant systems in rats organs after a year of training*	Wistar albino rats grouped in young control rats (YC), aged control rats (AC), and aged rats-training (AT)	Comparison of baseline samples between AT in swimming (T: 1 h/day for 5 days/week for 1 year) with YC and AC	Lung tissue	PT: >SOD activity and >GSH-Px in AT in comparison to AC; no difference of [TBARs] between the same groups
Lee et al. 2013 [123]	Administration of a ginseng intestinal metabolite (IH901) and exercise-induced oxidative stress in trained rat*	Sprague-Dawley rats divided into resting control (RC), training control (EC), resting with IH901 consumption, or exercise with IH901 consumption groups	Training was carried out during 8 weeks on a treadmill; two weeks with 0% inclination and 25 cm/sec; then 2 weeks with 10% and 30 cm/sec; then 4 weeks with 15% and 35 cm/sec	Lung tissue	PT: ↑ TBARs and ↑ protein carbonyls in EC versus RC
Menegali et al. 2009 [124]	Therapeutic effects of physical exercise on histological and oxidative stress markers in animals exposed to cigarette smoke*	Old C57BL-6 mice divided into control (C), training (T), cigarette smoke (CS), and cigarette smoke plus training (CS+E) groups	Training groups swam for 10 min/day during one habituation week; then they performed a swimming program 5 days/week for 8 weeks	Lung tissue	PT: ↑ SOD and ↑ CAT activity in E versus C
Olivo et al. 2014 [125]	Moderate aerobic exercise training prior to *Streptococcus pneumoniae* infection influences pulmonary inflammatory responses*	BALB/c mice divided into sedentary untreated (SU), sedentary infected (SI), aerobic trained untreated (ATU), and aerobic trained infected groups (ATI)	Comparison between SU and ATU during 4 weeks after an individual maximal exercise capacity test was performed (0.1 km/h every 2.5 min, 25% inclination); training was for 60 min/day, 5 days/wk for 4 wk at 50% of the maximal speed	BALF and lung tissue	PT: ↑ CuZn-SOD and ↑ Mn-SOD expression in lung parenchyma of ATU versus SU after an individual maximal exercise capacity test

(b) Continued.

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Reis Gonçalves et al. 2012 [15]	Chronic aerobic exercise on pulmonary inflammation, cytokine, and antioxidant enzymes in animal model of acute pulmonary damage*	Trained BALB/c mice	Comparison of samples before and after a low intensity training on treadmill (T: 50% of MS for 60 min/d, 3 d/week for 5 weeks)	BALF, EB, and lung tissue	PT: with no changes in leukocytes, [IL-6], [IL-10], nor [TNF-α] in BALF; with no changes in [NO] in EB; ↑ expression of IL-6 and Mn-SOD in the lung, but no changes of activity of GSH-Px and GR in the lung
Toledo et al. 2012 [126]	Regular physical exercise in an experimental mouse model exposed to cigarette smoke*	C57BL/6 mice divided into control mice (C), trained (T), exposed to cigarette smoke (Sk), and Sk plus T (Sk+T)	Comparison of baseline samples in T at moderate intensity on treadmill (T: 50% MS for 60 min/d, 5 d/week for 24 weeks)	BALF and lung tissue	PT: <[ROS] in BALF of En compared to C; >GSH-Px activity, but not of Mn-SOD nor CuZn-SOD in lungs of T compared to C; with no changes in the expression of IL-1ra, TNF-α, and IL-10 between T and C
Yang 2011 [127]	Chronic exercise and expression of cytokines related to inflammation in the lung tissue	Old male Sprague-Dawley rats, group with trained rats (T) and sedentary control rats (C)	Comparison of baseline samples between rats trained on treadmill (T: 25 m/min for 120 min/day for 1 week) and control rats	Lung tissue	>expression of mRNA for TNF-α and IL-4 and <expression of mRNA for IFN-γ of group T versus C

BALF: bronchoalveolar lavage fluid; BW: body weight; DEP: diesel exhaust particles; DNA: deoxyribonucleic acid; EB: exhaled breath; 8-OH-dG: 8-hydroxydeoxyguanosine; GR: glutathione reductase; GSH: glutathione reduced; GSH-Px: glutathione peroxidase; IFN-γ: interferon gamma; IL-1ra, IL-4, IL-6, or IL-10: interleukin-1ra, interleukin-4, interleukin-6, or interleukin-10; LPO: lipid peroxidation; MDA: malondialdehyde; MS: maximal speed; mRNA: messenger RNA; MS: maximal speed; NF-$\kappa\beta$/p65: factor nuclear kappa-β/p65; NO: nitric oxide; NO_2^-: nitrite; ROS: reactive oxygen species; SOD: superoxide dismutase; CuZn-SOD: copper-zinc-superoxide dismutase; Mn-SOD: manganese-superoxide dismutase; TBARs: thiobarbituric acid reactive substances; TNF-α: tumor necrosis factor-alpha. In "*Oxidative or inflammatory main results,*" PE: postexercise and PT: posttraining. In "*Aim,*" *the effect of exercise was not the primary object.

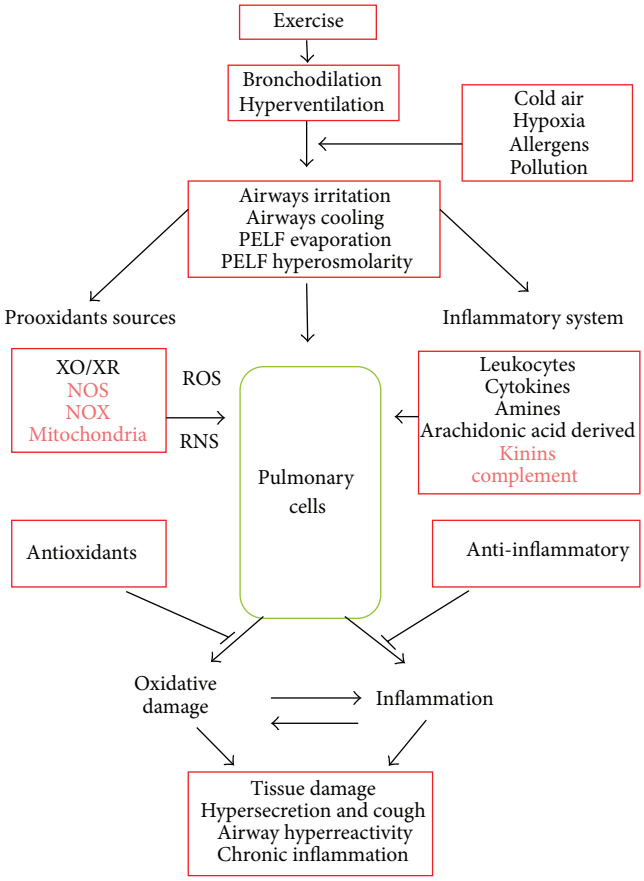

Figure 1: Proposed mechanisms related to the process of oxidative damage and pulmonary inflammation induced by exercise. Once the exercise starts the pulmonary ventilation increases and favors bronchodilation. This cools the airways, and also the part of PELF evaporates with subsequent increase of osmolarity and irritation appears. This activates the generating sources of free radicals and the inflammatory system. As a result of the foregoing, oxidative damage and a concomitant inflammatory process are potentially generated at pulmonary cell level; this may involve tissue damage, the increase of cough and the increased secretion of mucus, and the appearance of bronchoreactive phenomena and in the case that this stimulus is repeated (chronic exercise) to establish a process of chronic inflammation and remodeling of pulmonary tissue, particularly in the airways. This process is exacerbated when the exercise is performed in the presence of environmental conditions such as cold and hypoxia, in environments where pollen is abundant or in presence of contaminants (water/air). In red color the prooxidants sources and the parts of the inflammatory system that have not been studied are both appreciated. XO/XR = xanthine-oxidase/dehydrogenase; NOS = nitric oxide synthase; NOX = NADPH oxidase; PELF = pulmonary epithelial lining fluid.

shown controversial results. In swimmers, Bonsignore et al. [57] reported a decreased eNO after 5 km (~179 min) in slightly chlorinated pool; when performing the same test at the sea no changes were observed in this pair but the same distance was maintained at the sea. In other studies, also a decreased eNO after exercise has been observed in healthy subjects [64, 70, 88, 91]. However, in youngsters not trained in swimming, Carbonnelle et al. [58] found increases of eNO after swimming 2 sessions of ~1300 m in 45 min in a pool sanitized with electrical process (nonchlorinated water). Also, De Gouw et al. [61] found an increased eNO in healthy subjects after cycling for 6 min using dry air, while ventilation was kept constant in 40–50% of his or her predicted maximal voluntary ventilation (35 × FEV_1). Other studies showed no changes in the eNO after exercise; Font-Ribera et al. [65] found no differences in eNO concentrations in pool swimmers; the same occurred with eNO in swimmers after an exercise of 45 min [81] and in healthy subjects after either cycloergometer [66, 94] or treadmill incremental exercise test [80].

Through the exhaled breath condensate (EBC) analysis, to observe the oxidative effects of the moderate acute exercise, Nowak et al. [79] subjected a group of healthy subjects to a submaximal exercise on cycloergometer during ~6 min; they found no changes in H_2O_2 and thiobarbituric acid reactive substances (TBARs). Araneda et al. [46] found no changes of H_2O_2 in EBC after three maximal cycle ergometries of 1 min in elite cyclists carried out at 670 and 2160 masl, but malondialdehyde (MDA) was higher at 2160 meters. Marek et al. [72], in two submaximal cycle ergometries to 60 W (~7 min) and 120 W (~5 min), and later in maximal exercise (~13 min), found no differences in H_2O_2 concentration in EBC [73]; however, in both studies, increases were found in the flow of formed H_2O_2 after exercise. On the same prooxidant, Mercken et al. [76] found an increase after maximal cycle ergometry in healthy subjects, with increments of 10 w/min, but they did not find any differences in subjects with chronic obstructive pulmonary disease after exercise. However, in another study they found no differences in H_2O_2 when healthy subjects performed a cycle ergometry with one leg (40% P_{max}) during 20 min [77]. Marek et al. [74] found that, after 50 min of high intensity running developed at ~18°C and ~−15°C of environmental temperature, the concentration and production rates of H_2O_2 in EBC were higher when the exercise was carried out in a cold environment. Recently an increase in H_2O_2 and nitrite concentrations and correlations between both metabolites in the EBC of 21 and 42.2 km race participants were found. Also in this study, while nitrite increased in EBC, plasmatic nitrite showed no modifications and no correlations between these variables, which suggests a probable localized origin of this process [53].

Until now, only two studies have determined one of the potential sources of prooxidants; thus, it has been described as an increment of xanthine oxidase activity in the pulmonary homogenate of rats that performed strenuous exercise (~15 min) on a treadmill (20 m/min), besides MDA and NO [111]. Likewise, Huang et al. [110] observed an increase of the activity of xanthine oxidase and lung MDA in older rats after running on a treadmill until fatigue, during ~63 min at 70% VO_2max. Prigol et al. [115] and Akil et al. [105] found increases in TBARs in rats that swam for 20 min and 30 min, respectively, while Reddy et al. [114] found increases in MDA in rats with a vitamin E deficient diet that swam until fatigued. Also in rats, increases of TBARs after swimming during ~2.5 h until fatigue were found [106]. The same result was found in pulmonary homogenates of untrained rats which

swam until exhaustion [119]. A strenuous exercise protocol of ~66 min (80–85% VO_2max) showed no changes in TBARs in rats [107].

In healthy horses, no differences were observed in isoprostane 8-epi-PGF2α of supernatant of bronchoalveolar lavage fluid (BALF) after 50 min of running [13]. An increment of carbonyls in the lungs of rats was observed by Radák et al. [113] after an exercise till exhaustion on the treadmill. However, after an hour of a moderate intensity run in young and old rats, no changes were observed in the lung carbonyls [109].

With regard to the pulmonary antioxidant enzymes, after an hour of acute moderate exercise protocols on treadmills, young rats' lungs showed an increase in the activity of enzymes superoxide dismutase (SOD) of the type CuZn-SOD, Mn-SOD, of the catalase (CAT), without changes in the glutathione peroxidase (GSH-Px). The mRNA expression for these enzymes did not show differences [109]. Lin et al. [111] found an increase in SOD and glutathione reductase (GR) activity with no changes in CAT and GSH-Px activity in rats that ran at 30 m/min and 10% slope until fatigued. Finally, acute and prolonged exercise (more than an hour) at 80–85% VO_2max showed no changes in the activity of GSH-Px and SOD [107]. In acute exercise protocols, using swimming, Reddy et al. [114] found an increase in SOD and glutathione transferase (GST), while mild decreases in GSH-Px activity were observed in rats that swam until fatigued. Prigol et al. [115] found increase in CAT activity in rats that swam for 20 min. In rats that exercise for an hour, Terblanche [116] found increased CAT activity without differences between males and females. In rats 18 months old, Huang et al. [110] described an increase of SOD activity and the maintenance of levels of CAT, GSH-Px, and GR after 51 min on treadmill at 70% of VO_2max. Strenuous exercise increased the activity of GSH-Px, with no changes in GR [119]. In a report of Al-Hashem et al. [106], rats that exercised until fatigue decreased the activity of SOD and CAT.

Acute exercise has also altered the levels of nonenzymatic antioxidants; an increase of uric acid has been described, with no changes in total glutathione, in GSH, and in GSSG in BALF, after 50 min of incremental exercise in healthy horses [13]. In a study of rats that ran during ~81 min at 70–75% VO_2max until fatigue, no variations were found in the homogenized lung GSH [111]. In rats that swam until fatigue (~2.5 h), no differences were found at 600 m of altitude, but there was a decrease of GSH levels at 2270 meters [106]; in this same report, it was found that supplementation with nonenzymatic antioxidants such as VitC (20 mg/kg) and VitE (20 mg/kg), a single dose one hour before starting the exercise, decreases pulmonary lipid peroxidation and SOD and CAT activities increases, in both altitudes. Additionally, supplementation shows higher levels of GSH compared to animals not treated in altitude [106].

Thus, the increase in lung prooxidants and its consequences (lipid peroxidation) due to acute exercise appear to be related to the high intensity and duration of the effort, in terms of either minute ventilation or oxygen consumption, and are enhanced by a hostile environment (hypoxia, pollution, cold, etc.). However, a mainly enzymatic antioxidant adaptive response is still controversial. In contrast, the use of vitamin reducers (C and E) allows the antioxidant capacity to be increased and oxidative damage to be controlled (see Tables 1(a) and 1(b)).

5. Pulmonary Redox Balance and Chronic Exercise

In a first study of pulmonary prooxidants and chronic exercise, Carraro et al. [97] found no differences in eNO of child swimmers (trained 1 h/week during 6 months). Martin et al. [102] observed no differences in eNO of athletes based in pool and not based in pool exposed to pool environment during 5 and 0.5 h/week, respectively. For oxidative damage, Heinicke et al. [47] found a tendency towards increase of 8-isoprostanes in the EBC of biathletes who trained at 2800 meters during 6 weeks (4–6 h/d with 1 d/weeks of rest), which included extensive cross-country skiing, strength training, and shooting technique training.

In a model of physical training of rats, which jogged in 3 months a total of 24 sessions of 20 min/d at 60% VO_2max, no differences were found in pulmonary carbonyls, nitrite, or TBARs [121]. After 24 weeks of training at 50% V_{max} for 60 min/d for 5 d/week, ROS decreased in BALF and no changes of increase were found in pulmonary 8-isoprostanes in trained mice [126]. Using the same load and frequency as before, the levels of eNO and MDA were not altered in lung homogenates of rats trained during 5 weeks [15]. However, during the 8 weeks of training in rats that swam with a 2% of additional body weight during ~50–80 min, an increment of pulmonary carbonyls and MDA was observed [119]. Gündüz et al. [122] found increases of TBARs in older rats (21 months) versus young rats (9 months), without any variations between old rats which were either trained or untrained in swimming during 12 months 1 h/d for 5 d/week. Altan et al. [117] found increases in MDA in rats trained at 3000 meters of altitude (120 min/d for 4 d/week during 9 weeks) compared to sedentary control rats and the ones not trained maintained at sea or height level. In Sprague-Dawley rat that was trained during 8 weeks on a treadmill, an increase in pulmonary TBARs and protein carbonyls was observed [123]. Regarding oxidative stress on nucleic acids, Asami et al. [118] found increases in 8-hydroxydeoxyguanosine in rats after a forced race on treadmill for five weeks in daily sessions with a gradual increase in the time of 30–90 min.

The chronic exercising has also had as a subject of study the potential changes of the expression/activity of the enzymes and nonenzymes pulmonary antioxidant. Likewise, Reis Gonçalves et al. [15] found an increase in the lung Mn-SOD expression of mice subjected to five weeks of training at moderate intensity (60 min/d in 3 d/wk); however, no changes were observed in the GSH-Px, GR, GST, and CAT activities. In another study, Olivo et al. [125] observed an increased expression in pulmonary CuZn-SOD and Mn-SOD postmaximal exercise test of trained mice during 4 weeks at 50% of the maximal speed on treadmill. Altan et al. [117]

found increases of SOD activity after nine weeks of progressive training in a normobaric environment (5 to 30 min/d for 4 d/week), with no differences with a trained group at 3000 meters of altitude. da Cunha et al. [121] observed a higher pulmonary CAT activity in the ones trained on a treadmill during 12 weeks at 60% VO_2max (20 min/d), compared to control rats. In another study, Menegali et al. [124] found an increase of the CAT and SOD activity in lung of trained rat in swimming during 8 weeks. In mice trained on a treadmill for 24 weeks at 50% V_{max} (60 min/d and 5 d/week) increases of GSH-Px were observed without changes of expression of CuZn-SOD, Mn-SOD, and Ec-SOD, studied in sections of pulmonary tissue [126]. In another study, older animals of 21 months that were trained for a year (1 h/d and 5 d/week) had a greater amount of SOD in comparison to control rats of their same age and to young rats. No differences were found in CAT activities, while GSH-Px had a greater activity than a group of their same age [122]. Finally, Aydin et al. [119] observed a decrease in the concentrations of GSH and an increase of GSH-Px activity in pulmonary homogenates of rats, after eight weeks of swimming with overload and progressive weekly time increment (50–80 min).

This reflects the fact that oxidative stress induced by chronic pulmonary exercise in animals is closely associated with high-intensity protocols, but not with those of moderate intensity (see Table 1(b)). However, when moderate chronic exercise was executed while at high altitude, both human and animals presented pulmonary oxidative damage (see Tables 1(b) and 2(b)). In contrast, antioxidant adaptation seems to be more closely related to the animal training time, with an increase in the activity of SOD and CAT in the medium term and the expression of SOD in the short term (see Table 2(b)).

6. Acute Exercise-Induced Lung Inflammation

In horses, Kirschvink et al. [13] found no cellular count variation in BALF after 50 minutes of exercise. In runners' sputum of 10 km (~35.4 min), 12 km (~46.1 min), and 21 km (~89.1 min) a trend of increasing polymorphonuclear neutrophils (PMNs) in samples of induced sputum was found [40]. In the same direction, Bonsignore et al. [56] reported a higher percentage of PMNs in induced sputum, compared to values previous to exercise and an increase in these cells after the marathon (~179 min). Also in induced sputum of runners, Denguezli-Bouzgarrou et al. observed in 2006 [62] and 2007 [63] an increase of PMNs after 60 minutes of moderate racing. In the latter study, higher concentrations of histamine, interleukin-8 (IL-8), LTB_4, and LTE4 were also detected, subsequent to acute exercise during the precompetitive phase versus the competitive phase [63]. Chimenti et al. [5], in a 20-kilometer race (~90 min), reported an increase in IL-8 in the supernatant. Races in smaller time frames (~18 min) showed no changes in the amount of PMNs in induced sputum [93]. In rowers, after a short test of high intensity (1000 m in ~3 min), there was a trend towards an increase of epithelial cells and a positive association between the pulmonary ventilation/body weight (L/kg) and macrophages in induced sputum [78]. In swimmers, increases in lymphocytes and eosinophils and a decrease in macrophages were observed in induced sputum, after a 5 km race in the ocean (hypertonic environment) in relation to the same test performed in an open pool with low concentration of chlorine. However, there is no evidence of the increase in inflammatory cell activation [57]. In a chlorinated pool, in high performance swimmers, no changes were observed in the cellular composition of the induced sputum and the pH in EBC after 45 min at moderate intensity [81]. Larsson et al. [32] found an increase of granulocytes and macrophages in subjects that performed one hour of exercise, on a treadmill, at −23°C, without IL-8 changes in BALF samples. Derivatives of arachidonic acid have been studied in three works; thus, in a maximum acute exercise of approximately 12 min, increases in E_2 prostaglandin and B_2 thromboxane in EBC after exercise were found in men [83]. The leukotrienes in EBC were studied by Bikov et al. [54]; thus, after an eight-minute test on a treadmill no differences in the concentration of cysteinyl leukotrienes were found in normal people. In a test of 4 km of cycling with a 12% hill sloping during ~7 min, an increase of leukotriene B4 in BALF of athletes was found in comparison to the control subjects [67]. Also in EBC, Zietkowski et al. [95] found no changes in high sensitive C-reactive protein after 9 minutes of cycle-ergometry at 85% of HR_{max} in healthy subjects.

The pH in EBC (EBC_{pH}) is a potential marker of pulmonary inflammation that has been used in pathologies that have this condition. In acute exercise, the results have been variable; thus, Marek et al. [73] did not find differences after an exercise until fatigue (~13 min) in EBC_{pH} of amateur athletes. Bikov et al. [55] did not observe changes in the EBC_{pH} of healthy subjects after exercise, while there are other reports that show increases in pH after outdoor exercise [128] and after low-intensity (60% HR_{max}) exercise (~30 min) in nonathlete healthy subjects [84]. In races up to 10 km, no changes have been reported up to 80 min after the race, in both amateur runners [52] and physically active runners [53]. However, there are inverse correlations between changes in prooxidants and changes of EBC_{pH} [53]. In distances that exceed 21 and 42 km, ~101 min and ~246 min, respectively, an acute decreasing trend of EBC_{pH} was observed [52]. However, in an animal study conducted in horses, the group of Cathcart et al. [108] found an increase in EBC_{pH} after running 1.6 km.

In summary, the majority of published papers demonstrate the infiltration of inflammatory cells (macrophages or granulocytes) after acute exercise in humans. A factor that probably influences this is the duration of the exercise, as the increase in PMNs was found only in protocols involving longer periods (see Table 1(a)). Cellular infiltration was found to be due to cold or chlorine. The role of exercise training is difficult to assess, given that the studies were conducted almost exclusively in trained subjects. We must add to this the reported changes in soluble inflammatory mediators. As a whole, these could be an expression of an asymptomatic acute inflammatory process similar to that observed in other tissues (muscle tissue). This would happen in a self-limiting way whenever the necessary conditions of time, environmental factors, and intensity are encountered.

7. Chronic Exercise-Induced Lung Inflammation

Studies in animals have shown that training during 120 min/d for a week on treadmill at 25 m/min increases the expression of mRNA to tumor necrosis factor-alpha (TNF-α) together with promoting a decrease of interferon gamma in pulmonary tissue samples [127]. Chimenti et al. [120] trained mice at moderate intensity for 6 weeks (5 d/week), showing leukocyte infiltration in the airway. At this level of epithelia, an increase of apoptosis and a decrease of the ciliated cells were also observed. In mice that trained 60 min/d to 50% V_{max} for 24 weeks (5 d/week), no variation was observed in the number of macrophages in BALF, but it was possible to see a decrease of the capacity of these cells to form free radicals [126]. However, it is possible that the elaboration of training programs at moderate intensity (66% VO_2max) generates a reduction of the inflammatory response after the completion of ischemia and pulmonary reperfusion, which was evidenced as a decrease of the release of interleukin 1β and tumor necrosis factor-alpha (TNF-α) at plasmatic level in a model performed in rats [129]. An analogous result was described by Toledo et al. [126], who did not find differences in TNF-α, interleukin 10, monocyte chemotactic protein, and interleukin 1 receptor antagonist, quantified in lung sections of mice, after training to 50% V_{max} for 1 h/day, 5 days per week, for 24 weeks.

In studies conducted in humans, it has been reported that the participation in a long distance race training program over the course of a year generates a persistent inflammatory process with no apparent clinical repercussion and an increase in PMNs and in IL-8 concentrations, leukotriene E_4, and histamine in the supernatant of induced sputum samples [130]. Subjects who participated in high performance athletic training in sessions of 1 h/day for 10 days, interspersed with rest 5 days, had lower pH values in EBC compared to healthy control subjects [98]. The same result in this parameter was reported in runners by Greenwald et al. [128]. In the same direction, in amateur runners (~50 km/week) low levels of pH were reported compared to values of healthy control subjects [52]. High performance pool swimmers showed no differences in basal inflammatory parameters when compared with non-pool-based athletes; however, the analysis of the subgroup of athletes that had a positive result in the voluntary hyperventilation test (exercise-induced bronchial *hyperreactivity* indicator) presented a higher concentration of eNO and a higher count of eosinophils and of epithelial cells when compared to the group that had negative results on this test [102]; among other factors, this could be related to the number of years of practice of pool swimming, since no differences in eNO, in EBC pH, and in cellularity of induced sputum in adolescents were found when compared to normal subjects [131]. Elite swimmers, who trained between 800 and 3380 km/year, had more eosinophils and PMNs in induced sputum compared to nonathlete control subjects [99]. The cessation of the training for 3 months of swimmers decreases eosinophils and lymphocytes in induced sputum compared to active swimmers (~1870 km/year) [100]. The comparison between healthy athletes who are swimmers and others who are engaged in land exercise has shown an increased number of PMNs in induced sputum samples [96]; the same comparison showed no differences in PMNs and eosinophils in induced sputum [102]. Chronic inflammation can be associated with pulmonary epithelial damage; thus, increases of clear cell protein (CC16) in plasma of swimmers who trained during 20 weeks in a chlorinated pool have been reported [132].

In skiers, who trained 435 h/year, increase of lymphocytes and mast cells has been found, with no differences in the concentration of TNF-α and myeloperoxidase in BALF compared to nonathlete control subjects [103]. Karjalainen et al. [101] reported, through the study of bronchial biopsies, an increase in neutrophils, eosinophils, macrophages, and T lymphocytes in elite skiers (435 h/year) compared to healthy control subjects, along with air tract remodeling indicators as an increase in collagen I and collagen III deposits in the submucosa, a hyperplasia of racket cells, and a higher expression of type 5 mucin. The use of anti-inflammatories (800 micrograms/day of budesonide) by cross-country elite skiers (~427 h/year) during 20 weeks did not generate differences regarding the placebo (~468 h/year) in the cellularity (PMNs, macrophages, lymphocytes, eosinophils, and mast cells), studied in BALF and in endobronchial biopsy [104].

In summary, animal models of physical training show increases of soluble inflammatory mediators, which include TNF-α. Human studies have focused on subjects who have greater contact with irritants in the airway due to the specificity of their sport, whether runners (large ventilation volumes), skiers (cold), or swimmers (chlorine gas in the pool room). In these subjects, permanent tissue infiltration of granulocytes, macrophages, and lymphocytes has been observed. Evidence of these changes has been found in both noninvasive samples, such as induced sputum, and in biopsies in the bronchial region. At the same time, an increased presence of soluble proinflammatory substances has been reported. Overall, this suggests that these athletes in particular may suffer from persistent changes in tissue (chronic inflammation and airway remodeling) that have been associated with pulmonary symptoms and functional changes (see the bottom of Figure 1).

8. Oxidative Damage and Inflammation, Relations, and Potential Effects

The generation of prooxidant substances and the establishment of tissue oxidative damage are closely associated with inflammatory processes; thus, inflammatory cells are a known source of prooxidants derived from both oxygen and nitrogen [133]. At the same time, the increase of prooxidants has been involved in the intracellular signaling which leads to inflammatory cell activation, increased secretion of soluble mediators of inflammation [134], endothelial activation, and also increased expression of adhesion molecules and endothelial permeability [135]. This relation implies that, in many situations, the increase of prooxidants participates in the activation of inflammation and vice versa, demonstrating the close relationship between both phenomena [134].

The establishment of both oxidative damage and inflammation in the lungs has been involved in the origin/evolution of various pathological states; for example, both phenomena are a fundamental part of adult respiratory distress [136], asthma [137], chronic obstructive pulmonary disease [138], pulmonary hypertension [139], and viral infectious processes [140]. In the lungs, the relationship between oxidative changes and inflammation has rarely been studied as a main goal, but it is presumed that, in view of the studies conducted in other organs, it must be closely related. This is particularly important in subjects practicing sport, as both inflammation damage and oxidative damage have been implicated in the pathogenesis of phenomena of high prevalence in athletes such as rhinitis, bronchial hyperreactivity, asthma, and airway remodeling [27, 141]; so, most respiratory symptoms (coughing, wheezing, breathlessness, and chest tightness) in endurance athletes such as cross-country skiers are known [142]. In addition, cross-country skiers show a presence of PMNs and lymphocytes infiltration in the airways [101]. This phenomenon can also be extrapolated to other endurance athletes [143] such as marathon runners, cyclists, and swimmers, the latter of which are also exposed to the chlorine in swimming pools, which could be one of the main factors inducing increased eosinophils and leukocytes in the sputum.

9. Methods for the Study of Lung Inflammation/Oxidative Damage by Exercise

The study of the oxidative/inflammatory damage in the lungs is challenging due to both anatomic functional limitations and the limitations of currently applied techniques. Current evidence on this topic focuses primarily on the study of lung diseases, while studies on the effect of exercise as a trigger effect of this phenomenon in healthy people are scarce. Summarizing what is known to date for the species analyzed, the determinations made and the samples obtained are shown in Tables 1 and 2. Lung tissue microenvironment has challenged developers of study methodologies, so, although systemic markers have been proposed (CC16, surfactant proteins A and B, and Krebs von den Lungen-6), they do not yet have sufficient capacity to indicate minor damage, which implies that the processes of the lung itself cannot always be ascertained. For this reason, it is preferable to test samples originated from the lung; those currently under study are exhaled breath (whether direct or condensate), fluids (BALF, induced sputum, and nasal lavage), and cells and portions of whole tissue (biopsies, tissue homogenates, and cut pieces of tissue). Unfortunately, today there is still much controversy regarding the interpretation of the results obtained with these methods. In relation to oxidative/inflammatory exercise phenomenon, in animals, exhaled breath [112], lung tissue homogenates [113, 114, 117, 118, 120, 121, 127], bronchoalveolar lavage [121, 126], and lung tissue sections [126] have been used. In humans, most methods are focused on noninvasive methods and, among these, the induced sputum is the most widely used [40, 56, 57, 62, 63, 78, 81, 93, 96, 99, 100, 102, 144]. Another sample studied corresponds to exhaled breath, which was analyzed whether directly [56, 57, 59, 65, 71, 75, 81, 89, 97, 102] or after being condensed at low temperature [46, 53, 65, 72–74, 77, 79, 81, 83, 84, 128, 139]. Very few studies have used bronchoalveolar lavage [32, 103, 104] and lung tissue obtained by endobronchial biopsy [101, 104].

10. Discussion

In summary, we found that in acute exercise (see Tables 1(a) and 2(a)) there is more evidence of changes in cellularity (predominantly granulocytes) when it (was) is a prolonged high-intensity exercise. This change was not so evident in animals; however, this should be resolved in further studies because it is a parameter measured recently in this population. Long-term of acute moderate exercise (>60 min) in humans stimulated an increase of pulmonary inflammatory mediators (IL-8, LTB_4, and LTE_4). Now, regarding prooxidants, a systematic increase in humans is observed after more than thirty minutes of exercise. It is noteworthy that, in acute exercise in animals, reports of an increase in lung lipid peroxidation are the majority, while it has not been observed in humans, except for intense exercise at high altitudes. This may be partially explained by the techniques used: while tissue samples were analyzed in animals, EBC samples were analyzed in humans; in another aspect, the change with greater support in relation to the enzymatic activity corresponds to the maintenance or decreased levels of GSH-Px and to the increase in SOD.

With regard to chronic exercise (training) and its effects (see Tables 1(b) and 2(b)), the number of studies is still very small, but there is a tendency observed, seen in humans, towards changes in cellularity compatible with chronic inflammation of the airways, particularly in subjects exposed to cold and chlorine. In animals, changes in pulmonary cellularity (leukocyte infiltration) were observed in only one study [120]. For soluble inflammatory mediators, in animals the scientific evidence has shown an increase in the concentration of these substances (IL4, IL6, and mRNA TNF-α) subsequent to chronic exercise. The oxidative damage was observed in animals following moderate chronic exercise (>4 sem), specifically in older rats, and cold or altitude environment. In humans, only one study showed oxidative damage by altitude training [45, 47]. With regard to enzymatic antioxidants, a tendency towards higher levels in SOD and GSH-Px is observed in humans. As for nonenzymatic antioxidants, only one study showed a decrease in the concentration of pulmonary GSH in trained rats [119].

The problem requires further study to clarify numerous questions in order to have a more definitive overview; thus, several challenges for researchers in the field have arisen. Likewise, the activity of the sources of production of free radicals in the lung (mitochondria, xanthine oxidase, NADH oxidase, and NOS) should be studied and the knowledge of the status of antioxidant systems, particularly in humans, where there are no records available, should be improved. Regarding inflammatory parameters, the study of soluble mediators of inflammation should be extended; in addition, the effect of both substances with antioxidant and

anti-inflammatory effect should be explored. Furthermore, it is necessary to generate research projects which explore the parameters of oxidative/inflammatory mechanisms simultaneously in order to establish the interrelation mechanisms between both processes. It is also necessary to characterize the effect of time and intensity of performed exercise, the role of environmental conditions, and the level of training of the subjects on oxidative damage/lung inflammation by exercise. Finally, to advance the resolution of this problem, it is urgent to improve the technical conditions to allow obtaining representative samples of lung environment in its different compartments, and it is also necessary for these methods to be noninvasive and contribute to monitoring the athletes.

Conflict of Interests

The authors have no conflict of interests to declare.

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References

[1] L. Rokitzki, E. Logemann, A. N. Sagredos, M. Murphy, W. Wetzel-Rothl, and J. Keul, "Lipid peroxidation and antioxidative vitamins under extreme endurance stress," *Acta Physiologica Scandinavica*, vol. 150, no. 5, pp. 149–158, 1994.

[2] J. M. McBride, W. J. Kraemer, T. Triplett-McBride, and W. Sebastianelli, "Effect of resistance exercise on free radical production," *Medicine and Science in Sports and Exercise*, vol. 30, no. 1, pp. 67–72, 1998.

[3] D. P. Jones, "Redefining oxidative stress," *Antioxidants & Redox Signaling*, vol. 8, no. 9-10, pp. 1865–1879, 2006.

[4] U. K. Şentürk, F. Gündüz, O. Kuru et al., "Exercise-induced oxidative stress leads hemolysis in sedentary but not trained humans," *Journal of Applied Physiology*, vol. 99, no. 4, pp. 1434–1441, 2005.

[5] L. Chimenti, G. Morici, A. Paternò et al., "Bronchial epithelial damage after a half-marathon in nonasthmatic amateur runners," *The American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 298, no. 6, pp. L857–L862, 2010.

[6] G. Lippi, F. Schena, G. L. Salvagno, R. Aloe, G. Banfi, and G. C. Guidi, "Foot-strike haemolysis after a 60-km ultramarathon," *Blood Transfusion*, vol. 10, no. 3, pp. 377–383, 2012.

[7] A. Matsumoto, S. R. Mason, T. Flatscher-Bader et al., "Effects of exercise and antioxidant supplementation on endothelial gene expression," *International Journal of Cardiology*, vol. 158, no. 1, pp. 59–65, 2012.

[8] L. A. Silva, C. B. Tromm, G. Da Rosa et al., "Creatine supplementation does not decrease oxidative stress and inflammation in skeletal muscle after eccentric exercise," *Journal of Sports Sciences*, vol. 31, no. 11, pp. 1164–1176, 2013.

[9] D. A. McCarthy and M. M. Dale, "The leucocytosis of exercise. A review and model," *Sports Medicine*, vol. 6, no. 6, pp. 333–363, 1988.

[10] G. Camus, J. Pincemail, M. Ledent et al., "Plasma levels of polymorphonuclear elastase and myeloperoxidase after uphill walking and downhill running at similar energy cost," *International Journal of Sports Medicine*, vol. 13, no. 6, pp. 443–446, 1992.

[11] A. M. Niess, F. Passek, I. Lorenz et al., "Expression of the antioxidant stress protein heme oxygenase-1 (HO-1) in human leukocytes: acute and adaptational responses to endurance exercise," *Free Radical Biology and Medicine*, vol. 26, no. 1-2, pp. 184–192, 1999.

[12] A. M. Niess, H.-H. Dickhuth, H. Northoff, and E. Fehrenbach, "Free radicals and oxidative stress in exercise—immunological aspects," *Exercise Immunology Review*, vol. 5, pp. 22–56, 1999.

[13] N. Kirschvink, N. Smith, L. Fiévez et al., "Effect of chronic airway inflammation and exercise on pulmonary and systemic antioxidant status of healthy and heaves-affected horses," *Equine Veterinary Journal*, vol. 34, no. 6, pp. 563–571, 2002.

[14] E. C. Gomes, V. Stone, and G. Florida-James, "Impact of heat and pollution on oxidative stress and CC16 secretion after 8 km run," *European Journal of Applied Physiology*, vol. 111, no. 9, pp. 2089–2097, 2011.

[15] C. T. Reis Gonçalves, C. G. Reis Gonçalves, F. M. de Almeida et al., "Protective effects of aerobic exercise on acute lung injury induced by LPS in mice," *Critical Care*, vol. 16, no. 5, article R199, 2012.

[16] J. H. Roum, R. Buhl, N. G. McElvaney, Z. Borok, and R. G. Crystal, "Systemic deficiency of glutathione in cystic fibrosis," *Journal of Applied Physiology*, vol. 75, no. 6, pp. 2419–2424, 1993.

[17] R. K. Brown, H. Wyatt, J. F. Price, and F. J. Kelly, "Pulmonary dysfunction in cystic fibrosis is associated with oxidative stress," *The European Respiratory Journal*, vol. 9, no. 2, pp. 334–339, 1996.

[18] S. A. Peters and F. J. Kelly, "Vitamin E supplementation in cystic fibrosis," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 22, no. 4, pp. 341–345, 1996.

[19] I. Rahman and W. MacNee, "Lung glutathione and oxidative stress: implications in cigarette smoke-induced airway disease," *The American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 277, no. 6, pp. L1067–L1088, 1999.

[20] I. Rahman and W. MacNee, "Oxidative stress and regulation of glutathione in lung inflammation," *The European Respiratory Journal*, vol. 16, no. 3, pp. 534–554, 2000.

[21] T. S. Hallstrand, E. Calenoff, J. W. Becker, W. R. Henderson Jr., and M. L. Aitken, "The role of allergy in manifestations of respiratory disease in adult cystic fibrosis," *Annals of Allergy, Asthma & Immunology*, vol. 92, no. 2, pp. 228–233, 2004.

[22] I. Rahman, S. K. Biswas, and A. Kode, "Oxidant and antioxidant balance in the airways and airway diseases," *European Journal of Pharmacology*, vol. 533, no. 1–3, pp. 222–239, 2006.

[23] J. M. Weiler, S. Bonini, R. Coifman et al., "American Academy of Allergy, Asthma & Immunology Work Group report: exercise-induced asthma," *The Journal of allergy and clinical immunology*, vol. 119, no. 6, pp. 1349–1358, 2007.

[24] J. P. Parsons, C. P. Baran, G. Phillips et al., "Airway inflammation in exercise-induced bronchospasm occurring in athletes without asthma," *The Journal of Asthma*, vol. 45, no. 5, pp. 363–367, 2008.

[25] F. Holguin and A. Fitzpatrick, "Obesity, asthma, and oxidative stress," *Journal of Applied Physiology*, vol. 108, no. 3, pp. 754–759, 2010.

[26] B. M. Fischer, E. Pavlisko, and J. A. Voynow, "Pathogenic triad in COPD: oxidative stress, protease-antiprotease imbalance, and inflammation," *International Journal of Chronic Obstructive Pulmonary Disease*, vol. 6, no. 1, pp. 413–421, 2011.

[27] K. D. Fitch, "An overview of asthma and airway hyperresponsiveness in Olympic athletes," *British Journal of Sports Medicine*, vol. 46, no. 6, pp. 413–416, 2012.

[28] K. J. Burnham, T. J. Arai, D. J. Dubowitz et al., "Pulmonary perfusion heterogeneity is increased by sustained, heavy exercise in humans," *Journal of Applied Physiology*, vol. 107, no. 5, pp. 1559–1568, 2009.

[29] K. C. Beck, B. D. Johnson, T. P. Olson, and T. A. Wilson, "Ventilation-perfusion distribution in normal subjects," *Journal of Applied Physiology*, vol. 113, no. 6, pp. 872–877, 2012.

[30] A. N. Freed and M. S. Davis, "Hyperventilation with dry air increases airway surface fluid osmolality in canine peripheral airways," *American Journal of Respiratory and Critical Care Medicine*, vol. 159, no. 4, part 1, pp. 1101–1107, 1999.

[31] E. R. McFadden Jr. and B. M. Pichurko, "Intraairway thermal profiles during exercise and hyperventilation in normal man," *The Journal of Clinical Investigation*, vol. 76, no. 3, pp. 1007–1010, 1985.

[32] K. Larsson, G. Tornling, D. Gavhed, C. Müller-Suur, and L. Palmberg, "Inhalation of cold air increases the number of inflammatory cells in the lungs in healthy subjects," *The European Respiratory Journal*, vol. 12, no. 4, pp. 825–830, 1998.

[33] A. Kendall, L. Hoffman-Goetz, M. Houston, B. MacNeil, and Y. Arumugam, "Exercise and blood lymphocyte subset responses: intensity, duration, and subject fitness effects," *Journal of Applied Physiology*, vol. 69, no. 1, pp. 251–260, 1990.

[34] D. C. Nieman, L. M. Johanssen, J. W. Lee, and K. Arabatzis, "Infectious episodes in runners before and after the Los Angeles Marathon," *Journal of Sports Medicine and Physical Fitness*, vol. 30, no. 3, pp. 316–328, 1990.

[35] G. W. Heath, E. S. Ford, T. E. Craven, C. A. Macera, K. L. Jackson, and R. R. Pate, "Exercise and the incidence of upper respiratory tract infections," *Medicine and Science in Sports and Exercise*, vol. 23, no. 2, pp. 152–157, 1991.

[36] D. C. Nieman, L. M. Onasch, and J. W. Lee, "The effects of moderate exercise training on nutrient intake in mildly obese women," *Journal of the American Dietetic Association*, vol. 90, no. 11, pp. 1557–1562, 1990.

[37] D. C. Nieman, D. A. Henson, G. Gusewitch et al., "Physical activity and immune function in elderly women," *Medicine and Science in Sports and Exercise*, vol. 25, no. 7, pp. 823–831, 1993.

[38] S. D. Anderson, R. E. Schoeffel, R. Follet, C. P. Perry, E. Daviskas, and M. Kendall, "Sensitivity to heat and water loss at rest and during exercise in asthmatic patients," *European Journal of Respiratory Diseases*, vol. 63, no. 5, pp. 459–471, 1982.

[39] S. D. Anderson and P. Kippelen, "Exercise-induced bronchoconstriction: pathogenesis," *Current Allergy and Asthma Reports*, vol. 5, no. 2, pp. 116–122, 2005.

[40] L. Chimenti, G. Morici, A. Paternò et al., "Environmental conditions, air pollutants, and airway cells in runners: a longitudinal field study," *Journal of Sports Sciences*, vol. 27, no. 9, pp. 925–935, 2009.

[41] P. L. Kinney, D. M. Nilsen, M. Lippmann et al., "Biomarkers of lung inflammation in recreational joggers exposed to ozone," *American Journal of Respiratory and Critical Care Medicine*, vol. 154, no. 5, pp. 1430–1435, 1996.

[42] C. C. Daigle, D. C. Chalupa, F. R. Gibb et al., "Ultrafine particle deposition in humans during rest and exercise," *Inhalation Toxicology*, vol. 15, no. 6, pp. 539–552, 2003.

[43] N. D. Magnani, T. Marchini, D. R. Tasat, S. Alvarez, and P. A. Evelson, "Lung oxidative metabolism after exposure to ambient particles," *Biochemical and Biophysical Research Communications*, vol. 412, no. 4, pp. 667–672, 2011.

[44] F. Farina, G. Sancini, C. Battaglia et al., "Milano summer particulate matter (PM10) triggers lung inflammation and extra pulmonary adverse events in mic," *PLoS ONE*, vol. 8, no. 2, Article ID e56636, 2013.

[45] S. Wittkopp, N. Staimer, T. Tjoa et al., "Mitochondrial genetic background modifies the relationship between traffic-related air pollution exposure and systemic biomarkers of inflammation," *PLoS ONE*, vol. 8, no. 5, Article ID e64444, 2013.

[46] O. F. Araneda, C. García, N. Lagos et al., "Lung oxidative stress as related to exercise and altitude. Lipid peroxidation evidence in exhaled breath condensate: a possible predictor of acute mountain sickness," *European Journal of Applied Physiology*, vol. 95, no. 5-6, pp. 383–390, 2005.

[47] I. Heinicke, A. Boehler, T. Rechsteiner et al., "Moderate altitude but not additional endurance training increases markers of oxidative stress in exhaled breath condensate," *European Journal of Applied Physiology*, vol. 106, no. 4, pp. 599–604, 2009.

[48] A. J. Carlisle and N. C. C. Sharp, "Exercise and outdoor ambient air pollution," *British Journal of Sports Medicine*, vol. 35, no. 4, pp. 214–222, 2001.

[49] I. S. Mudway and F. J. Kelly, "An investigation of inhaled ozone dose and the magnitude of airway inflammation in healthy adults," *American Journal of Respiratory and Critical Care Medicine*, vol. 169, no. 10, pp. 1089–1095, 2004.

[50] H. Adachi, P. H. Nguyen, R. Belardinelli, D. Hunter, T. Sung, and K. Wasserman, "Nitric oxide production during exercise in chronic heart failure," *American Heart Journal*, vol. 134, no. 2, pp. 196–202, 1997.

[51] P. Agostoni and M. Bussotti, "Exhaled nitric oxide and exercise performance in heart failure," *Archives of Physiology and Biochemistry*, vol. 111, no. 4, pp. 293–296, 2003.

[52] O. F. Araneda, A. J. Guevara, C. Contreras, N. Lagos, and F. J. Berral, "Exhaled breath condensate analysis after long distance races," *International Journal of Sports Medicine*, vol. 33, no. 12, pp. 955–961, 2012.

[53] O. F. Araneda, R. Urbina-Stagno, M. Tuesta et al., "Increase of pro-oxidants with no evidence of lipid peroxidation in exhaled breath condensate after a 10-km race in non-athletes," *Journal of Physiology and Biochemistry*, vol. 70, no. 1, pp. 107–115, 2014.

[54] A. Bikov, R. Gajdócsi, É. Huszár et al., "Exercise induced—exercise increases exhaled breath condensate cysteinyl leukotriene concentration in asthmatic patients," *Journal of Asthma*, vol. 47, no. 9, pp. 1057–1062, 2010.

[55] A. Bikov, G. Galffy, L. Tamasi et al., "Exhaled breath condensate pH decreases during exercise-induced bronchoconstriction," *Respirology*, vol. 19, no. 4, pp. 563–569, 2014.

[56] M. R. Bonsignore, G. Morici, L. Riccobono et al., "Airway inflammation in nonasthmatic amateur runners," *The American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 281, no. 3, pp. L668–L676, 2001.

[57] M. R. Bonsignore, G. Morici, L. Riccobono et al., "Airway cells after swimming outdoors or in the sea in nonasthmatic athletes,"

Medicine and Science in Sports and Exercise, vol. 35, no. 7, pp. 1146–1152, 2003.

[58] S. Carbonnelle, A. Bernard, I. R. Doyle, J. Grutters, and M. Francaux, "Fractional exhaled NO and serum pneumoproteins after swimming in a chlorinated pool," *Medicine and Science in Sports and Exercise*, vol. 40, no. 8, pp. 1472–1476, 2008.

[59] M. F. Chirpaz-Oddou, A. Favre-Juvin, P. Flore et al., "Nitric oxide response in exhaled air during an incremental exhaustive exercise," *Journal of Applied Physiology*, vol. 82, no. 4, pp. 1311–1318, 1997.

[60] E. Clini, L. Bianchi, M. Vitacca, R. Porta, K. Foglio, and N. Ambrosino, "Exhaled nitric oxide and exercise in stable COPD patients," *Chest*, vol. 117, no. 3, pp. 702–707, 2000.

[61] H. W. F. M. De Gouw, S. J. Marshall-Partridge, H. Van Der Veen, J. G. Van Den Aardweg, P. S. Hiemstra, and P. J. Sterk, "Role of nitric oxide in the airway response to exercise in healthy and asthmatic subjects," *Journal of Applied Physiology*, vol. 90, no. 2, pp. 586–592, 2001.

[62] M. Denguezli-Bouzgarrou, B. Sriha, I. Ben Cheikh, W. Ben Turkia, Z. Tabka, and A. Zbidi, "Effect of endurance exercise on airway cells in runners," *Science & Sports*, vol. 21, no. 2, pp. 99–100, 2006.

[63] M. Denguezli-Bouzgarrou, H. Ben Saad, I. Ben Chiekh, S. Gaied, Z. Tabka, and A. Zbidi, "Role of lung inflammatory mediators as a cause of training-induced lung function changes in runners," *Science & Sports*, vol. 22, no. 1, pp. 35–42, 2007.

[64] B. Evjenth, T. E. Hansen, and J. Holt, "Exhaled nitric oxide decreases during exercise in non-asthmatic children," *The Clinical Respiratory Journal*, vol. 7, no. 2, pp. 121–127, 2013.

[65] L. Font-Ribera, M. Kogevinas, J.-P. Zock et al., "Short-term changes in respiratory biomarkers after swimming in a chlorinated pool," *Environmental Health Perspectives*, vol. 118, no. 11, pp. 1538–1544, 2010.

[66] F. García-Río, M. Ramírez, O. Mediano et al., "Exhaled nitric oxide and airway caliber during exercise-induced bronchoconstriction," *International Journal of Sports Medicine*, vol. 27, no. 11, pp. 905–910, 2006.

[67] S. R. Hopkins, R. B. Schoene, W. R. Henderson, R. G. Spragg, T. R. Martin, and J. B. West, "Intense exercise impairs the integrity of the pulmonary blood-gas barrier in elite athletes," *American Journal of Respiratory and Critical Care Medicine*, vol. 155, no. 3, pp. 1090–1094, 1997.

[68] P. Kippelen, C. Caillaud, E. Robert, K. Masmoudi, and C. Préfaut, "Exhaled nitric oxide level during and after heavy exercise in athletes with exercise-induced hypoxaemia," *Pflugers Archiv*, vol. 444, no. 3, pp. 397–404, 2002.

[69] S. L. Lovell, H. Stevenson, I. S. Young et al., "Exhaled nitric oxide during incremental and constant workload exercise in chronic cardiac failure," *European Journal of Clinical Investigation*, vol. 30, no. 3, pp. 181–187, 2000.

[70] K. J. Mantione, T. Esch, and G. B. Stefano, "Detection of nitric oxide in exhaled human breath: exercise and resting determinations," *Medical Science Monitor*, vol. 13, no. 3, pp. MT1–MT5, 2007.

[71] A. Matsumoto, Y. Hirata, S.-I. Momomura et al., "Increased nitric oxide production during exercise," *The Lancet*, vol. 343, no. 8901, pp. 849–850, 1994.

[72] E. Marek, K. Mückenhoff, H. J. Streckert, G. Becher, and W. Marek, "Measurements of L-lactate and H_2O_2 in exhaled breath condensate at rest and mild to moderate exercise in young and healthy subjects," *Pneumologie*, vol. 62, no. 9, pp. 541–547, 2008.

[73] E. Marek, P. Platen, J. Volke, K. Mückenhoff, and W. Marek, "Hydrogen peroxide release and acid-base status in exhaled breath condensate at rest and after maximal exercise in young, healthy subjects," *European Journal of Medical Research*, vol. 14, supplement 4, pp. 134–139, 2009.

[74] E. Marek, J. Volke, K. Mückenhoff, P. Platen, and W. Marek, "Exercise in cold air and hydrogen peroxide release in exhaled breath condensate," *Advances in Experimental Medicine and Biology*, vol. 756, pp. 169–177, 2013.

[75] M. J. Maroun, S. Mehta, R. Turcotte, M. G. Cosio, and S. N. A. Hussain, "Effects of physical conditioning on endogenous nitric oxide output during exercise," *Journal of Applied Physiology*, vol. 79, no. 4, pp. 1219–1225, 1995.

[76] E. M. Mercken, G. J. Hageman, A. M. W. J. Schols, M. A. Akkermans, A. Bast, and E. F. M. Wouters, "Rehabilitation decreases exercise-induced oxidative stress in chronic obstructive pulmonary disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 172, no. 8, pp. 994–1001, 2005.

[77] E. M. Mercken, H. R. Gosker, E. P. Rutten et al., "Systemic and pulmonary oxidative stress after single-leg exercise in COPD," *Chest*, vol. 136, no. 5, pp. 1291–1300, 2009.

[78] G. Morici, M. R. Bonsignore, D. Zangla et al., "Airway cell composition at rest and after an all-out test in competitive rowers," *Medicine and Science in Sports and Exercise*, vol. 36, no. 10, pp. 1723–1729, 2004.

[79] D. Nowak, S. Kalucka, P. Bialasiewicz, and M. Król, "Exhalation of H_2O_2 and thiobarbituric acid reactive substances (TBARs) by healthy subjects," *Free Radical Biology & Medicine*, vol. 30, no. 2, pp. 178–186, 2001.

[80] P. Nadziakiewicz, P. Knapik, D. Ziora, and D. Nowak, "Moderate exercise decreases nitric oxide exhalation in patients with stable coronary artery disease," *Journal of Physiology and Pharmacology*, vol. 57, supplement 4, pp. 213–221, 2006.

[81] L. Pedersen, T. K. Lund, E. Mølgaard, S. A. Kharitonov, P. J. Barnes, and V. Backer, "The acute effect of swimming on airway inflammation in adolescent elite swimmers," *The Journal of Allergy and Clinical Immunology*, vol. 123, no. 2, pp. 502–504, 2009.

[82] S. Pogliaghi, J. A. Krasney, and D. R. Pendergast, "Effect of gravity on lung exhaled nitric oxide at rest and during exercise," *Respiration Physiology*, vol. 107, no. 2, pp. 157–164, 1997.

[83] J. M. Pucsok, I. Györe, K. Argay et al., "Effect of exercise on levels of cyclo-oxygenase mediators in exhaled breath condensate in elite athletes," *The Journal of Sports Medicine and Physical Fitness*, vol. 47, no. 2, pp. 223–227, 2007.

[84] M. Riediker and B. Danuser, "Exhaled breath condensate pH is increased after moderate exercise," *Journal of Aerosol Medicine: Deposition, Clearance, and Effects in the Lung*, vol. 20, no. 1, pp. 13–18, 2007.

[85] M. S. Riley, J. Pórszász, J. Miranda, M. P. K. J. Engelen, B. Brundage, and K. Wasserman, "Exhaled nitric oxide during exercise in primary pulmonary hypertension and pulmonary fibrosis," *Chest*, vol. 111, no. 1, pp. 44–50, 1997.

[86] G. Rolla, L. Mangiardi, C. Bucca et al., "Exhaled nitric oxide during exercise and dobutamine stress echocardiography in patients with mitral stenosis," *European Journal of Internal Medicine*, vol. 14, no. 3, pp. 166–171, 2003.

[87] H.-W. Shin, C. M. Rose-Gottron, D. M. Cooper, M. Hill, and S. C. George, "Impact of high-intensity exercise on nitric oxide exchange in healthy adults," *Medicine and Science in Sports and Exercise*, vol. 35, no. 6, pp. 995–1003, 2003.

[88] C. M. St Croix, T. J. Wetter, D. F. Pegelow, K. C. Meyer, and J. A. Dempsey, "Assessment of nitric oxide formation during exercise," *American Journal of Respiratory and Critical Care Medicine*, vol. 159, no. 4, part 1, pp. 1125–1133, 1999.

[89] A. Therminarias, M. F. Oddou, A. Favre-Juvin, P. Flore, and M. Delaire, "Bronchial obstruction and exhaled nitric oxide response during exercise in cold air," *European Respiratory Journal*, vol. 12, no. 5, pp. 1040–1045, 1998.

[90] G. Trolin, T. Anden, and G. Hedenstierna, "Nitric oxide (NO) in expired air at rest and during exercise," *Acta Physiologica Scandinavica*, vol. 150, no. 5, pp. 159–164, 1994.

[91] E. Tufvesson, H. Svensson, J. Ankerst, and L. Bjermer, "Increase of club cell (Clara) protein (CC16) in plasma and urine after exercise challenge in asthmatics and healthy controls, and correlations to exhaled breath temperature and exhaled nitric oxide," *Respiratory Medicine*, vol. 107, no. 11, pp. 1675–1681, 2013.

[92] S. Verges, J. Tonini, P. Flore, A. Favre-Juvin, P. Lévy, and B. Wuyam, "Exhaled nitric oxide in single and repetitive prolonged exercise," *Journal of Sports Sciences*, vol. 24, no. 11, pp. 1157–1163, 2006.

[93] T. J. Wetter, Z. Xiang, D. A. Sonetti et al., "Role of lung inflammatory mediators as a cause of exercise-induced arterial hypoxemia in young athletes," *Journal of Applied Physiology*, vol. 93, no. 1, pp. 116–126, 2002.

[94] Y. Yasuda, T. Itoh, M. Miyamura, and H. Nishino, "Comparison of exhaled nitric oxide and cardiorespiratory indices between nasal and oral breathing during submaximal exercise in humans," *The Japanese Journal of Physiology*, vol. 47, no. 5, pp. 465–470, 1997.

[95] Z. Zietkowski, R. Skiepko, M. M. Tomasiak-Lozowska, B. Mroczko, M. Szmitkowski, and A. Bodzenta-Lukaszyk, "Changes in high-sensitivity C-reactive protein in serum and exhaled breath condensate after intensive exercise in patients with allergic asthma," *International Archives of Allergy and Immunology*, vol. 153, no. 1, pp. 75–85, 2010.

[96] J. Belda, S. Ricart, P. Casan et al., "Airway inflammation in the elite athlete and type of sport," *British Journal of Sports Medicine*, vol. 42, no. 4, pp. 244–249, 2008.

[97] S. Carraro, M. F. Pasquale, M. Da Frè et al., "Swimming pool attendance and exhaled nitric oxide in children," *The Journal of Allergy and Clinical Immunology*, vol. 118, no. 4, pp. 958–960, 2006.

[98] J. M. Ferdinands, C. A. G. Crawford, R. Greenwald, D. Van Sickle, E. Hunter, and W. G. Teague, "Breath acidification in adolescent runners exposed to atmospheric pollution: a prospective, repeated measures observational study," *Environmental Health: A Global Access Science Source*, vol. 7, article 10, 2008.

[99] I. J. Helenius, P. Rytilä, T. Metso, T. Haahtela, P. Venge, and H. O. Tikkanen, "Respiratory symptoms, bronchial responsiveness, and cellular characteristics of induced sputum in elite swimmers," *Allergy*, vol. 53, no. 4, pp. 346–352, 1998.

[100] I. Helenius, P. Rytilä, S. Sarna et al., "Effect of continuing or finishing high-level sports on airway inflammation, bronchial hyperresponsiveness, and asthma: a 5-year prospective follow-up study of 42 highly trained swimmers," *The Journal of Allergy and Clinical Immunology*, vol. 109, no. 6, pp. 962–968, 2002.

[101] E.-M. Karjalainen, A. Laitinen, M. Sue-Chu, A. Altraja, L. Bjermer, and L. A. Laitinen, "Evidence of airway inflammation and remodeling in ski athletes with and without bronchial hyperresponsiveness to methacholine," *American Journal of Respiratory and Critical Care Medicine*, vol. 161, no. 6, pp. 2086–2091, 2000.

[102] N. Martin, M. R. Lindley, B. Hargadon, W. R. Monteiro, and I. D. Pavord, "Airway dysfunction and inflammation in pool-and non-pool-based elite athletes," *Medicine and Science in Sports and Exercise*, vol. 44, no. 8, pp. 1433–1439, 2012.

[103] M. Sue-Chu, L. Larsson, T. Moen, S. I. Rennard, and L. Bjermer, "Bronchoscopy and bronchoalveolar lavage findings in cross-country skiers with and without 'ski asthma'," *The European Respiratory Journal*, vol. 13, no. 3, pp. 626–632, 1999.

[104] M. Sue-Chu, E.-M. Karjalainen, A. Laitinen, L. Larsson, L. A. Laitinen, and L. Bjermer, "Placebo-controlled study of inhaled budesonide on indices of airway inflammation in bronchoalveolar lavage fluid and bronchial biopsies in cross-country skiers," *Respiration*, vol. 67, no. 4, pp. 417–425, 2000.

[105] M. Akil, U. Gurbuz, M. Bicer, I. Halifeoglu, A. K. Baltaci, and R. Mogulkoc, "Selenium prevents lipid peroxidation in liver and lung tissues of rats in acute swimming exercise," *Bratislavské Lekárske Listy*, vol. 116, no. 4, pp. 233–235, 2015.

[106] F. H. Al-Hashem, "Potential roles for vitamins E and C in combination in modulating exhaustive swimming and high altitudeassociated lung injury in rats," *Saudi Medical Journal*, vol. 33, no. 4, pp. 367–374, 2012.

[107] C. Caillaud, G. Py, N. Eydoux, P. Legros, C. Prefaut, and J. Mercier, "Antioxidants and mitochondrial respiration in lung, diaphragm, and locomotor muscles: effect of exercise," *Free Radical Biology & Medicine*, vol. 26, no. 9-10, pp. 1292–1299, 1999.

[108] M. P. Cathcart, S. Love, D. G. M. Sutton, R. J. M. Reardon, and K. J. Hughes, "The application of exhaled breath analysis in racing Thoroughbreds and the influence of high intensity exercise and ambient temperature on the concentration of carbon monoxide and pH in exhaled breath," *Veterinary Journal*, vol. 197, no. 2, pp. 318–323, 2013.

[109] H. Hatao, S. Oh-Ishi, M. Itoh et al., "Effects of acute exercise on lung antioxidant enzymes in young and old rats," *Mechanisms of Ageing and Development*, vol. 127, no. 4, pp. 384–390, 2006.

[110] C.-C. Huang, S.-C. Tsai, and W.-T. Lin, "Potential ergogenic effects of L-arginine against oxidative and inflammatory stress induced by acute exercise in aging rats," *Experimental Gerontology*, vol. 43, no. 6, pp. 571–577, 2008.

[111] W.-T. Lin, S.-C. Yang, K.-T. Chen, C.-C. Huang, and N.-Y. Lee, "Protective effects of L-arginine on pulmonary oxidative stress and antioxidant defenses during exhaustive exercise in rats," *Acta Pharmacologica Sinica*, vol. 26, no. 8, pp. 992–999, 2005.

[112] P. C. Mills, D. J. Marlin, E. Demoncheaux et al., "Nitric oxide and exercise in the horse," *The Journal of Physiology*, vol. 495, no. 3, pp. 863–874, 1996.

[113] Z. Radák, A. Nakamura, H. Nakamoto, K. Asano, H. Ohno, and S. Goto, "A period of anaerobic exercise increases the accumulation of reactive carbonyl derivatives in the lungs of rats," *Pflügers Archiv: European Journal of Physiology*, vol. 435, no. 3, pp. 439–441, 1998.

[114] K. V. Reddy, T. C. Kumar, M. Prasad, and P. Reddanna, "Pulmonary lipid peroxidation and antioxidant defenses during exhaustive physical exercise: the role of vitamin E and selenium," *Nutrition*, vol. 14, no. 5, pp. 448–451, 1998.

[115] M. Prigol, C. Luchese, and C. W. Nogueira, "Antioxidant effect of diphenyl diselenide on oxidative stress caused by acute physical exercise in skeletal muscle and lungs of mice," *Cell Biochemistry and Function*, vol. 27, no. 4, pp. 216–222, 2009.

[116] S. E. Terblanche, "The effects of exhaustive exercise on the activity levels of catalase in various tissues of male and female rats," *Cell Biology International*, vol. 23, no. 11, pp. 749–753, 1999.

[117] M. Altan, P. Atukeren, M. Mengi, G. Metin, L. Cakar, and K. Gumustas, "Influence of intermittent hypobaric exposure on SOD and TBARS levels in trained rats," *Chinese Journal of Physiology*, vol. 52, no. 2, pp. 106–112, 2009.

[118] S. Asami, T. Hirano, R. Yamaguchi, Y. Tsurudome, H. Itoh, and H. Kasai, "Effects of forced and spontaneous exercise on 8-hydroxydeoxyguanosine levels in rat organs," *Biochemical and Biophysical Research Communications*, vol. 243, no. 3, pp. 678–682, 1998.

[119] C. Aydin, F. Sonat, S. K. Sahin, I. T. Cangul, and G. Ozkaya, "Long term dietary restriction ameliorates swimming exercise-induced oxidative stress in brain and lung of middle-aged rat," *Indian Journal of Experimental Biology*, vol. 47, no. 1, pp. 24–31, 2009.

[120] L. Chimenti, G. Morici, A. Paternò et al., "Endurance training damages small airway epithelium in mice," *American Journal of Respiratory and Critical Care Medicine*, vol. 175, no. 5, pp. 442–449, 2007.

[121] M. J. da Cunha, A. A. da Cunha, G. K. Ferreira et al., "The effect of exercise on the oxidative stress induced by experimental lung injury," *Life Sciences*, vol. 92, no. 3, pp. 218–227, 2013.

[122] F. Gündüz, Ü. K. Şentürk, O. Kuru, B. Aktekin, and M. R. Aktekin, "The effect of one year's swimming exercise on oxidant stress and antioxidant capacity in aged rats," *Physiological Research*, vol. 53, no. 2, pp. 171–176, 2004.

[123] N.-J. Lee, J.-W. Lee, J.-H. Sung, Y.-G. Ko, S. Hwang, and J.-K. Kang, "Effects of administration of IH901, a ginsenoside intestinal metabolite, on muscular and pulmonary antioxidant functions after eccentric exercise," *Journal of Veterinary Science*, vol. 14, no. 3, pp. 249–256, 2013.

[124] B. T. Menegali, R. T. Nesi, P. S. Souza et al., "The effects of physical exercise on the cigarette smoke-induced pulmonary oxidative response," *Pulmonary Pharmacology & Therapeutics*, vol. 22, no. 6, pp. 567–573, 2009.

[125] C. R. Olivo, E. N. Miyaji, M. L. S. Oliveira et al., "Aerobic exercise attenuates pulmonary inflammation induced by *Streptococcus pneumoniae*," *Journal of Applied Physiology*, vol. 117, no. 9, pp. 998–1007, 2014.

[126] A. C. Toledo, R. M. Magalhaes, D. C. Hizume et al., "Aerobic exercise attenuates pulmonary injury induced by exposure to cigarette smoke," *The European Respiratory Journal*, vol. 39, no. 2, pp. 254–264, 2012.

[127] D. S. Yang, "Effects of strenuous exercise on expressions of inflammation-related cytokines of lung tissue in rats," *International Journal of Infectious Diseases*, vol. 15, pp. S106–S107, 2011.

[128] R. Greenwald, J. M. Ferdinands, and W. G. Teague, "Ionic determinants of exhaled breath condensate pH before and after exercise in adolescent athletes," *Pediatric Pulmonology*, vol. 44, no. 8, pp. 768–777, 2009.

[129] R. K. Mussi, E. A. Camargo, T. Ferreira et al., "Exercise training reduces pulmonary ischaemia-reperfusion-induced inflammatory responses," *The European Respiratory Journal*, vol. 31, no. 3, pp. 645–649, 2008.

[130] M. Denguezli, I. Ben Chiekh, H. Ben Saad, M. Zaouali-Ajina, Z. Tabka, and A. Zbidi, "One-year endurance training: effects on lung function and airway inflammation," *Journal of Sports Sciences*, vol. 26, no. 12, pp. 1351–1359, 2008.

[131] L. Pedersen, T. K. Lund, P. J. Barnes, S. A. Kharitonov, and V. Backer, "Airway responsiveness and inflammation in adolescent elite swimmers," *The Journal of Allergy and Clinical Immunology*, vol. 122, no. 2, pp. 322–327.e1, 2008.

[132] Á. Fernández-Luna, L. Gallardo, M. Plaza-Carmona et al., "Respiratory function and changes in lung epithelium biomarkers after a short-training intervention in chlorinated vs. ozone indoor pools," *PLoS ONE*, vol. 8, no. 7, Article ID e68447, 2013.

[133] S. Bréchard and E. J. Tschirhart, "Regulation of superoxide production in neutrophils: role of calcium influx," *Journal of Leukocyte Biology*, vol. 84, no. 5, pp. 1223–1237, 2008.

[134] H. Lum and K. A. Roebuck, "Oxidant stress and endothelial cell dysfunction," *American Journal of Physiology—Cell Physiology*, vol. 280, no. 4, pp. C719–C741, 2001.

[135] R. Scalia and A. M. Lefer, "In vivo regulation of PECAM-1 activity during acute endothelial dysfunction in the rat mesenteric microvasculature," *Journal of Leukocyte Biology*, vol. 64, no. 2, pp. 163–169, 1998.

[136] C. E. Cross, B. Frei, and S. Louie, "The adult respiratory distress syndrome (ARDS) and oxidative stress: therapeutic implications," *Advances in Experimental Medicine and Biology*, vol. 264, pp. 435–448, 1990.

[137] A. Ahmad, M. Shameem, and Q. Husain, "Relation of oxidant-antioxidant imbalance with disease progression in patients with asthma," *Annals of Thoracic Medicine*, vol. 7, no. 4, pp. 226–232, 2012.

[138] G. Fisher, D. D. Schwartz, J. Quindry et al., "Lymphocyte enzymatic antioxidant responses to oxidative stress following high-intensity interval exercise," *Journal of Applied Physiology*, vol. 110, no. 3, pp. 730–737, 2011.

[139] O. F. Araneda and M. Tuesta, "Lung oxidative damage by hypoxia," *Oxidative Medicine and Cellular Longevity*, vol. 2012, Article ID 856918, 18 pages, 2012.

[140] S. Selemidis, H. J. Seow, B. R. S. Broughton et al., "Nox1 oxidase suppresses influenza a virus-induced lung inflammation and oxidative stress," *PLoS ONE*, vol. 8, no. 4, Article ID e60792, 2013.

[141] L.-P. Boulet, "Cough and upper airway disorders in elite athletes: a critical review," *British Journal of Sports Medicine*, vol. 46, no. 6, pp. 417–421, 2012.

[142] K. Larsson, P. Ohlsen, L. Larsson, P. Malmberg, P.-O. Rydstrom, and H. Ulriksen, "High prevalence of asthma in cross country skiers," *British Medical Journal*, vol. 307, no. 6915, pp. 1326–1329, 1993.

[143] M. Sue-Chu, "Winter sports athletes: long-term effects of cold air exposure," *British Journal of Sports Medicine*, vol. 46, no. 6, pp. 397–401, 2012.

[144] L.-P. Boulet, H. Turcotte, J.-B. Langdeau, and M.-C. Bernier, "Lower airway inflammatory responses to high-intensity training in athletes," *Clinical and Investigative Medicine*, vol. 28, no. 1, pp. 15–22, 2005.

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Research Article

Exercise-Induced Changes in Caveolin-1, Depletion of Mitochondrial Cholesterol, and the Inhibition of Mitochondrial Swelling in Rat Skeletal Muscle but Not in the Liver

Damian Jozef Flis,[1,2] Robert Antoni Olek,[2] Jan Jacek Kaczor,[3] Ewa Rodziewicz,[2] Malgorzata Halon,[4] Jedrzej Antosiewicz,[5] Michal Wozniak,[6] Rosita Gabbianelli,[7] and Wieslaw Ziolkowski[2]

[1]*Department of Bioenergetics and Physiology of Exercise, Medical University of Gdansk, 80-210 Gdansk, Poland*
[2]*Department of Bioenergetics and Nutrition, Gdansk University of Physical Education and Sport, 80-336 Gdansk, Poland*
[3]*Department of Physiotherapy, Gdansk University of Physical Education and Sport, 80-336 Gdansk, Poland*
[4]*Department of Physiotherapy, Medical University of Gdansk, 80-210 Gdansk, Poland*
[5]*Department of Biochemistry, Gdansk University of Physical Education and Sport, 80-336 Gdansk, Poland*
[6]*Department of Medical Chemistry, Medical University of Gdansk, 80-210 Gdansk, Poland*
[7]*School of Pharmacy, University of Camerino, 62032 Camerino, Italy*

Correspondence should be addressed to Wieslaw Ziolkowski; wiech@gumed.edu.pl

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The reduction in cholesterol in mitochondria, observed after exercise, is related to the inhibition of mitochondrial swelling. Caveolin-1 (Cav-1) plays an essential role in the regulation of cellular cholesterol metabolism and is required by various signalling pathways. Therefore, the aim of this study was to investigate the effect of prolonged swimming on the mitochondrial Cav-1 concentration; additionally, we identified the results of these changes as they relate to the induction of changes in the mitochondrial swelling and cholesterol in rat skeletal muscle and liver. Male Wistar rats were divided into a sedentary control group and an exercise group. The exercised rats swam for 3 hours and were burdened with an additional 3% of their body weight. After the cessation of exercise, their quadriceps femoris muscles and livers were immediately removed for experimentation. The exercise protocol caused an increase in the Cav-1 concentration in crude muscle mitochondria; this was related to a reduction in the cholesterol level and an inhibition of mitochondrial swelling. There were no changes in rat livers, with the exception of increased markers of oxidative stress in mitochondria. These data indicate the possible role of Cav-1 in the adaptive change in the rat muscle mitochondria following exercise.

1. Introduction

The significance of the exercise-induced depletion in the mitochondrial cholesterol pool is still not fully understood. The reduction in cholesterol in rat heart mitochondria, observed after exercise, is related to the inhibition of mitochondrial swelling; however, this change does not influence the mitochondrial bioenergetics and oxidative stress. [1, 2]. It has been proposed that this phenomenon may be involved in the protection mechanism of mitochondria during exercise or during other stressful conditions [2].

Mitochondrial swelling [3], a significant mediator of cell death, results from the opening of a mitochondrial permeability transition pore (mPTP) [4, 5]. Important factor implicated in mPTP regulation is intracellular calcium. Physiological stimuli, such as physical exercise, that cause an increase of cytosolic free Ca^{2+} or the release of Ca^{2+} from the endoplasmic reticulum invariably induce mitochondrial

Ca^{2+} uptake, with a rise of mitochondrial matrix-free Ca^{2+}. Hence, mitochondria accumulate Ca^{2+} and efficiently control the spatial and temporal shape of cellular Ca^{2+} signals. This situation exposes mitochondria to the hazards of Ca^{2+} overload, which can lead to the opening of the mPTP. Persistent mPTP opening is followed by depolarization, with Ca^{2+} release, cessation of oxidative phosphorylation, matrix swelling with inner membrane remodelling, and, eventually, outer membrane rupture with release of cytochrome c and other apoptogenic proteins [6].

Caveolins (Cav) are essential components of caveolae, which are plasma membrane invaginations that demonstrate reduced fluidity; this reflects an accumulation of cholesterol [7]. Cav proteins bind cholesterol, and Cav's ability to move between cellular compartments helps to control intracellular cholesterol fluxes [7–9]. The first member of the Cav family (including Cav-1, Cav-2, and Cav-3), Cav-1, is a 22 kDa protein of 178 amino acids that plays an essential role in the regulation of the cellular cholesterol metabolism of various signalling molecules (Src-like kinases, H-Ras, endothelial nitric-oxide synthase, and G proteins); these molecules lead to effective communication between extracellular signals and the interior of the cell [10]. Cav-1 is predominately found in terminally differentiated cells, such as adipocytes, endothelia and smooth muscle cells, and type I pneumocytes [11]; additionally, it has been identified in skeletal muscles [12] and the liver [13, 14].

Cav-1 deficiency is related to impaired mitochondrial function, free cholesterol accumulation in mitochondrial membranes, increased membrane condensation, a reduction of the respiratory chain, and accumulation of reactive oxygen species [15].

However, to our knowledge, there are no data explaining the mechanism responsible for the cholesterol depletion in mitochondria following exercise. Therefore, the aim of this study was to investigate the effect of prolonged swimming on mitochondrial Cav-1 and cholesterol concentrations as well as the induction of changes in mitochondrial swelling in rat skeletal muscles and livers. Based on our previous data [2], we hypothesize that both exercise-induced changes in the mitochondrial swelling and mitochondrial cholesterol levels will occur with concomitant increases in Cav-1 concentrations in the skeletal muscle mitochondria but not in the liver.

2. Materials and Methods

2.1. Materials/Reagents. All chemicals were purchased from Sigma (St. Louis, MO, USA), with the exception of BSA (Merck, Darmstadt, Germany). The cholesterol assay kit was generously donated by CHEMA Diagnostica (Monsano, Italy).

2.2. Animals

2.2.1. Animal Care. Male Wistar rats ($n = 12$) weighing 400–450 g were housed in an environmentally controlled room (23 ± 1°C with a 12 h light-dark cycle); the rats received standard rat chow and water ad libitum. Experiments were conducted in accordance with the principles of the UK legislation and were approved by the Local Ethics Committee of the Gdansk Medical University (consent no. 13/2007). The animals were anesthetized by intraperitoneal injection of ketamine and xylazine (90 and 10 $mg \cdot kg^{-1}$, resp.). Skeletal muscles (m. quadriceps) and livers of the anesthetized animals were rapidly removed, weighed, and immersed in an ice-cold isolation buffer (0.07 M sucrose, 0.23 M mannitol, 0.003 M HEPES, and 0.001 M EGTA; pH 7.4 for the liver and 0.17 M sucrose, 0.075 M KCl, 0.05 M Tris-base, 0.001 M KH_2PO_4, 0.005 M $MgCl_2$, and 0.001 M EGTA; pH 7.4 for muscle) for crude mitochondria isolation.

2.2.2. Exercise Protocol. The rats were prepared for the experiments and exercise tests using the previously described methods [2, 16]. The rats were randomly divided into sedentary controls (C) and long-lasting endurance exercise (E) groups. Before the experiments, the animals in the E group were trained to reduce the stress of swimming. Each day during the preparatory procedure, the rats swam for 30 min in water at 35°C. On the first day, the rats swam without any additional weight. On the second, third, and fourth days, the rats swam burdened with 1, 2, and 3% of their body weight, respectively, on their tails. On the fifth day, exercise testing was performed in the E group of rats, which consisted of 3 h of prolonged swimming in 35°C water, burdened with 3% of their body weight. The rats were euthanized (as described in Section 2.2.1) immediately after completing their protocol. As we previously demonstrated, the temperature of the water and the preparatory procedure prior to the exercise test did not influence the studied parameters [1].

2.3. Isolation of Rat Mitochondria

2.3.1. Rat Liver Mitochondria (RLM). The liver mitochondria were isolated, as previously described by Broekemeier et al. [17] with slight modification. The liver was rapidly removed, weighed, and placed in an ice-cold mitochondrial buffer A (mM: 230 mannitol, 70 sucrose, 3 HEPES, and 0.1 EGTA, pH 7.4); it was then rinsed three times. The liver was then minced with scissors and homogenized using a Teflon pestle homogenizer in buffer B (buffer B = buffer A + 0.1% BSA). The homogenate was centrifuged at 700 ×g for 10 min. The supernatant was decanted and centrifuged at 7000 ×g for 10 min. The pellet was resuspended in 40 mL of suspension (buffer C = buffer A without 1 mM EGTA) and centrifuged again at 7000 ×g for 10 min. This step was repeated three times. The final mitochondrial pellet was resuspended in 0.5 mL of buffer C.

2.3.2. Rat Quadriceps Mitochondria (RQM). The skeletal muscle mitochondria were isolated, as previously described by Fontaine et al. [18] with slight modifications. The quadriceps muscle was dissected from the surrounding connective tissue, rapidly removed, trimmed clean of visible connective tissue, weighed, and placed in 10 mL of ice-cold mitochondrial isolation buffer A (mM: 150 sucrose, 75 KCl, 50 Tris base, 1 KH_2PO_4, 5 $MgCl_2$, 1 EGTA, and 0.2% BSA, pH 7.4). Muscles were minced with scissors, incubated for 1 min with Nagarse protease (10 mL of isolation buffer per 1 g of tissue,

supplemented with Nagarse (0.2 mg mL^{-1})), and homogenized using a Teflon pestle homogenizer. The homogenate volume was increased to 40 mL by adding cold isolation buffer, which was then centrifuged at 700 ×g for 10 min. The supernatant was decanted and centrifuged at 10 000 ×g for 10 min. The pellet was resuspended in 40 mL of suspension buffer B (mM: 250 sucrose, 10 Tris-base, and 0.05 EGTA, pH 7.4) and centrifuged at 10 000 ×g for 10 min. The final mitochondrial pellet was resuspended in buffer B (0.25 μL of buffer B per 1 g of muscle mass).

All steps were performed at 4°C.

2.4. Swelling of the Mitochondria. The measurement of RLM and RQM swelling was spectrophotometrically performed, as previously described for the liver by Crouser et al. [19] and for the skeletal muscle by Csukly et al. [20]. Liver mitochondria were incubated at 25°C in a medium containing (in mM) 230 mannitol, 70 sucrose, 2.0 K_2HPO_4, and 3.0 HEPES, pH 7.4. RQM were incubated at the same temperature in a medium containing (in mM) 250 sucrose, 10 MOPS, 10 K_2HPO_4, and 10 Tris-HCl, pH 7.3.

$CaCl_2$ (100 μM) was used as a mitochondrial permeability transition pore (mPTP) opening inducer and cyclosporin A (CSA, 1 μM) was used as an mPTP opening inhibitor. 1 mg of mitochondria was added to the appropriate buffer, which was supplemented with 5 mM succinate and 1 μM rotenone.

The swelling curves were recorded at 540 nm. Cuvettes, containing the mitochondrial suspension, were kept at 25°C.

The greater decrease in absorbance is related to greater susceptibility of mitochondria to calcium chloride-induced mitochondrial swelling. Such deenergized and inactivated mitochondria are rapidly and physiologically less resistant to opening mPTP, which precedes the process of cell death.

2.5. Cholesterol Estimation. Total lipids were extracted from the mitochondria normalized to the mitochondrial protein concentration. The cholesterol content in extracts was measured using the CHEMA Diagnostica cholesterol assay kit with a standard cholesterol solution as a reference. Total lipids extraction and cholesterol measurement procedures were performed as previously described [21].

2.6. Determination of Caveolin-1 Concentration in Liver and Quadriceps Crude Mitochondria. The concentration of caveolin-1 was measured using the Cloud-Clone Corporation Caveolin-1 ELISA Kit (Category number SEA214Ra) according to the manufacturer's instructions.

2.7. Oxidative Stress Parameters in Rat Liver and Quadriceps Crude Mitochondria. The carbonyl groups [22] and lipid dienes [23] were measured in crude mitochondria isolated from the livers and quadriceps of the C and E rat groups. The values of the carbonyl groups are expressed as nmol·mg of protein-1, and the lipid dienes are expressed as an oxidation index 233/215.

2.8. Protein Measurement. The protein content was measured using the method described by Lowry et al. [24] with BSA as the standard.

2.9. Data Analysis. Statistical analyses were performed using a software package (Statistica v. 10.0, StatSoft Inc., Tulsa, OK, USA). The results are expressed as the mean ± standard error (SE). The differences between the means were tested using the unpaired Student's t-test. The results were statistically significant when $P < 0.05$.

3. Results

3.1. Mitochondrial Cholesterol Content of Quadriceps Muscles and Livers in the Control and Exercised Groups of Rats. The prolonged swimming protocol caused a significant drop in the cholesterol level in crude mitochondria of the quadriceps muscle. The mitochondria isolated from the skeletal muscle of exercised rats had approximately 84% of the cholesterol compared with control rats (Figure 1(a)). No significant changes in the cholesterol levels were observed in crude mitochondria isolated from liver (C versus E group, Figure 1(b)).

3.2. Swelling of Mitochondria. We determined whether mitochondrial cholesterol depletion was associated with mitochondrial swelling. The calcium chloride-induced mitochondrial swelling was significantly lower in the rat quadriceps muscle of mitochondria isolated after exercise when compared with the control (Figure 2(a)). The inhibitory effects of 1 μM CSA (data not shown) were observed. No changes in the mitochondrial swelling were observed in the liver mitochondria (Figure 2(b)).

3.3. Content of the Caveolin-1 in Control and Exercised Quadriceps and Liver Mitochondria. To verify whether the depletion of the mitochondrial cholesterol level after exercise is related to changes in Cav, the concentration of Cav-1 was determined in crude mitochondria of the C and E groups.

Prolonged swimming caused a significant increase in the content of Cav-1 in the quadriceps of crude mitochondria (Figure 3(a)). No changes in the Cav-1 content were observed in crude mitochondria isolated from liver (Figure 3(b)). The relationship between cholesterol and Cav-1 content in the crude quadriceps muscle mitochondria is presented in Figure 4.

3.4. Prolonged Swimming Induces Oxidative Stress in Livers but Not in Skeletal Muscles. To verify that exercise was able to induce oxidative stress, protein carbonyl groups and lipid dienes were measured in the liver and quadriceps muscle crude mitochondria of the C and E groups of rats. Significantly higher oxidative stress parameters were only observed in the E group of crude mitochondria in the liver (Table 1).

4. Discussion

In the present study, we demonstrate that adaptive responses to prolonged exercise are related to increased content of Cav-1 in isolated quadriceps muscle mitochondria; moreover, there is concomitant depletion of mitochondrial cholesterol and increased resistance to Ca^{2+} induced swelling. We did not observe similar changes in rat liver mitochondria from the

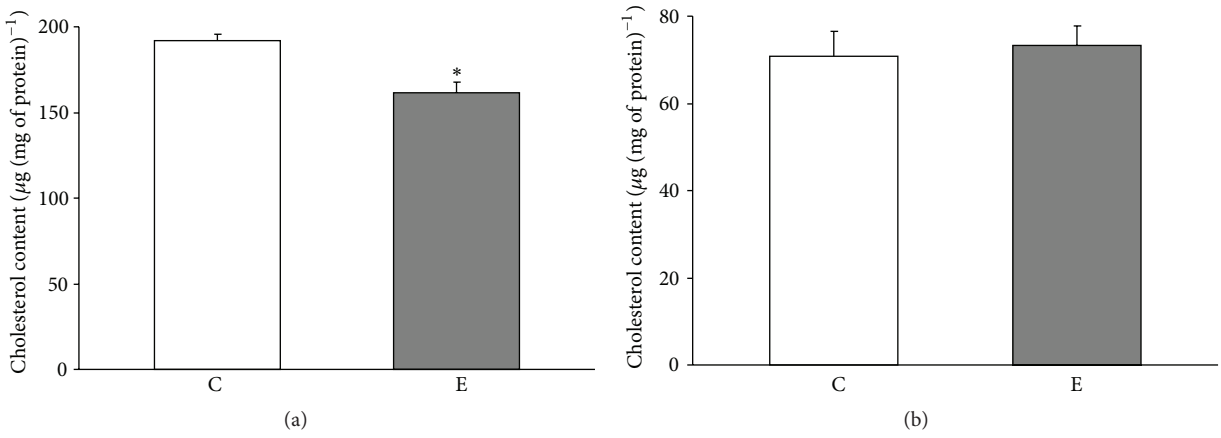

Figure 1: Mitochondrial cholesterol levels in controlled and exercised animals. Cholesterol was measured in crude mitochondria isolated from the quadriceps (a) and liver (b). The data are presented as the means ± SE. * There was a significant difference between the E (exercise) versus C (control) in the Quadriceps mitochondria $^{*}P = 0.001$ versus the control rats ($n = 6$ in each group).

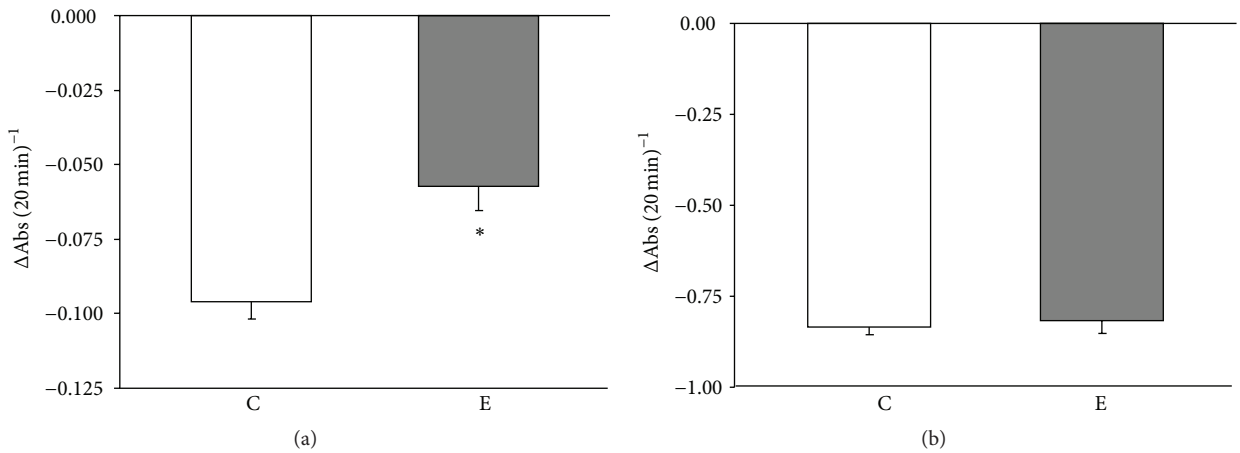

Figure 2: Mitochondrial swelling in controlled and exercised animals. The calcium chloride-induced mitochondrial swelling was spectrophotometrically assessed in the quadriceps (a) and liver (b) crude mitochondria. The data are presented as the means ± SE. * There was a significant difference between the E (exercise) versus C (control) in the quadriceps mitochondria $^{*}P = 0.002$ versus the control rats ($n = 6$ in each group).

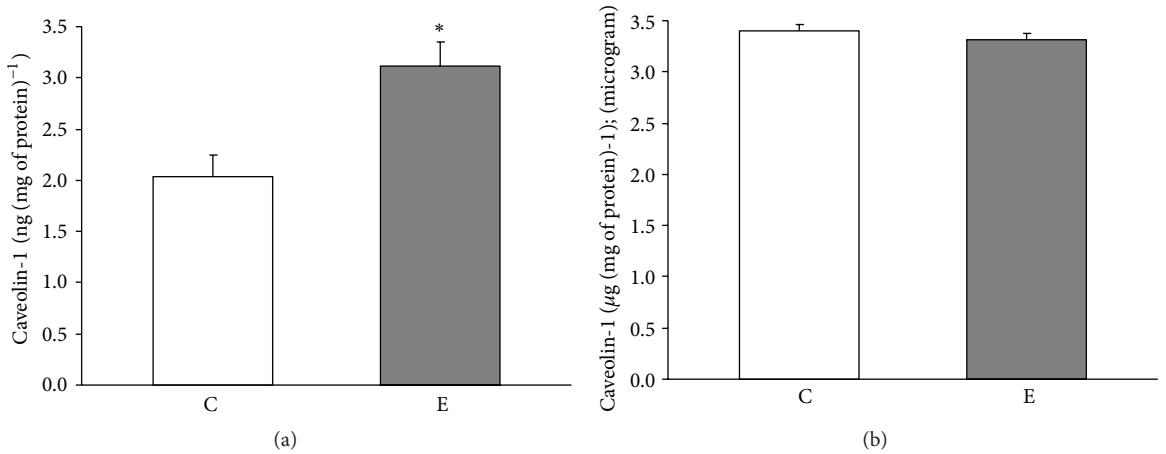

Figure 3: Mitochondrial caveolin-1 levels in controlled and exercised animals. Caveolin-1 level was measured in crude mitochondria isolated from the quadriceps (a) and liver (b). The data are presented as the means ± SE. * There was a significant difference between the E (exercise) versus C (control) in the quadriceps mitochondria $^{*}P = 0.006$ versus the control rats ($n = 6$ in each group).

Table 1: Oxidative stress parameters (carbonyl groups and lipid dienes) in Quadriceps and Liver mitochondria of the C and E groups of rats.

	Quadriceps mitochondria		Liver mitochondria	
	C	E	C	E
Carbonyl groups (nmol (mg of protein)$^{-1}$)	1.460 ± 0.08	1.364 ± 0.12	1.125 ± 0.07	1.732 ± 0.12*
Lipid dienes (oxidation index (233/215))	0.172 ± 0.003	0.169 ± 0.004	0.230 ± 0.005	0.260 ± 0.005$^{\#}$

The mean values ± SE are expressed as nmol (mg of protein)$^{-1}$ (for carbonyl groups) and oxidation index 233/215 (lipid dienes). $^{*}P = 0.0008$, $^{\#}P = 0.0007$. The E (exercise) group is significantly different from the C (control) group ($n = 6$ in each group).

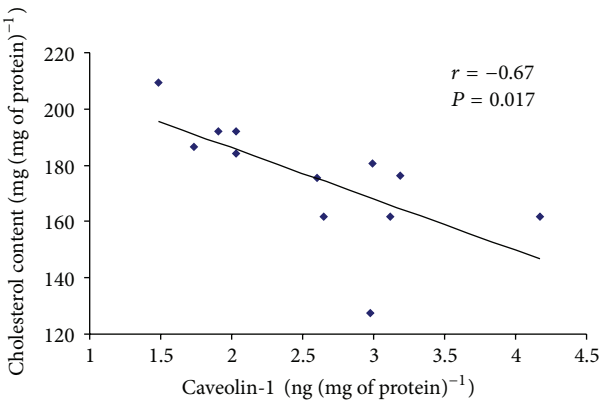

Figure 4: Correlation between caveolin-1 and cholesterol levels in quadriceps crude mitochondria. A Pearson product-moment correlation coefficient was computed to assess the relationship between the caveolin-1 and cholesterol level in the crude mitochondria isolated from the quadriceps. There was a large, negative correlation between the two variables, $r = -0.67$, $P = 0.017$.

exercised group, but we did observe an increase in the level of protein carbonyl groups and lipid dienes in mitochondria (markers of oxidative stress). There was an inverse correlation between mitochondrial cholesterol and Cav-1 levels in rat tissues after exercise. The data confirm our initial hypothesis that both exercise-induced changes in mitochondrial swelling and the level of cholesterol are related to increases in Cav-1 concentrations in skeletal muscle mitochondria but not in the liver. We suspect that this phenomenon refers exclusively to the contractile tissues, such as heart and skeletal muscles. The finding implies that a reduction in the cholesterol levels and an increased mitochondria sensitivity to swelling are not deleterious; furthermore, these alterations may be a result of changes in Cav-1 concentrations in the mitochondria. As we have previously indicated, these modifications are related to a dynamic physiological process that may help mitochondria adapt to stress conditions [1, 2]. Changes in mitochondrial cholesterol levels in exercised rats were previously observed in heart tissues but not in skeletal muscles. Another novelty of this study is an indication of the possible role of Cav-1 in the aforementioned process.

4.1. The Effect of Swimming Exercise on Cav-1 and Cholesterol Levels in Liver and Skeletal Muscle Mitochondria. To our knowledge, there are no data that show changes in cholesterol and Cav-1 levels in liver and skeletal muscle mitochondria after exercise. However, Park and colleagues showed that there are changes in the Cav-1 concentrations in skeletal muscles [25] and in rat brain tissues following training [26]. The Cav-1 in the skeletal muscle is a fibre and exercise-specific [12].

Our data demonstrate that prolonged swimming reduced cholesterol levels and increased Cav-1 concentrations in rat skeletal muscle mitochondria. We did not observe such changes in the hepatic mitochondria, which leads us to suspect that this effect is specific to contractile tissues. Furthermore, there were exercise-induced changes in the mitochondrial Cav-1 and cholesterol content in skeletal muscles but not in the liver; this may have resulted from different responses in these tissues during oxidative stress.

Recently, we reported that modifications in mitochondrial function depend on the degree of depletion of cholesterol. This may be comparable to the changes observed after exercise. These changes did not impair the mitochondrial bioenergetics; furthermore, the changes positively inhibited the mitochondrial swelling, likely by remodelling the lipid microdomains [2]. However, in the *in vitro* study, adding 4% methyl-beta-cyclodextrin (a known cholesterol chelator) resulted in a lowering of the cholesterol in mitochondria below the range observed after exercise; additionally, there was a decline in mitochondrial function, which led to changes in the mitochondrial configuration state [27]. Thus, an appropriate level of cholesterol in mitochondria is necessary to maintain their function. Both too high and too low mitochondrial cholesterol levels lower the oxidative phosphorylation. This may be the main reason that mitochondrial dysfunction is related to a decrease in ATP synthesis, ATP hydrolysis (respiration in mitochondria), and impaired ADP/ATP exchange (for review, see [28]).

It has been shown that mitochondria are cholesterol-sensitive organelles, but little is known about the regulation of their cholesterol influx/efflux [29]. Cav-1, which is expressed abundantly in lipid rafts of many cells and organelles membranes [10], seems to be an excellent candidate for the cholesterol regulator in mitochondria. In fact, this protein plays an important role in the cholesterol transport inside the cell and in organelles, particularly mitochondria [15]. Bosch and coworkers demonstrated, *in vitro*, that mouse embryonic fibroblast cells from Cav-1$^{-/-}$ mice exhibited free cholesterol accumulation in mitochondrial membranes, increased membrane condensation, reduced efficiency of the respiratory chain, and intrinsic antioxidant defences when compared with wild-type mice [15]. It was shown that changes in the level of cholesterol in the mitochondria are dependent on the presence of caveolin; this, therefore, affects the function of mitochondria and antioxidant status of the

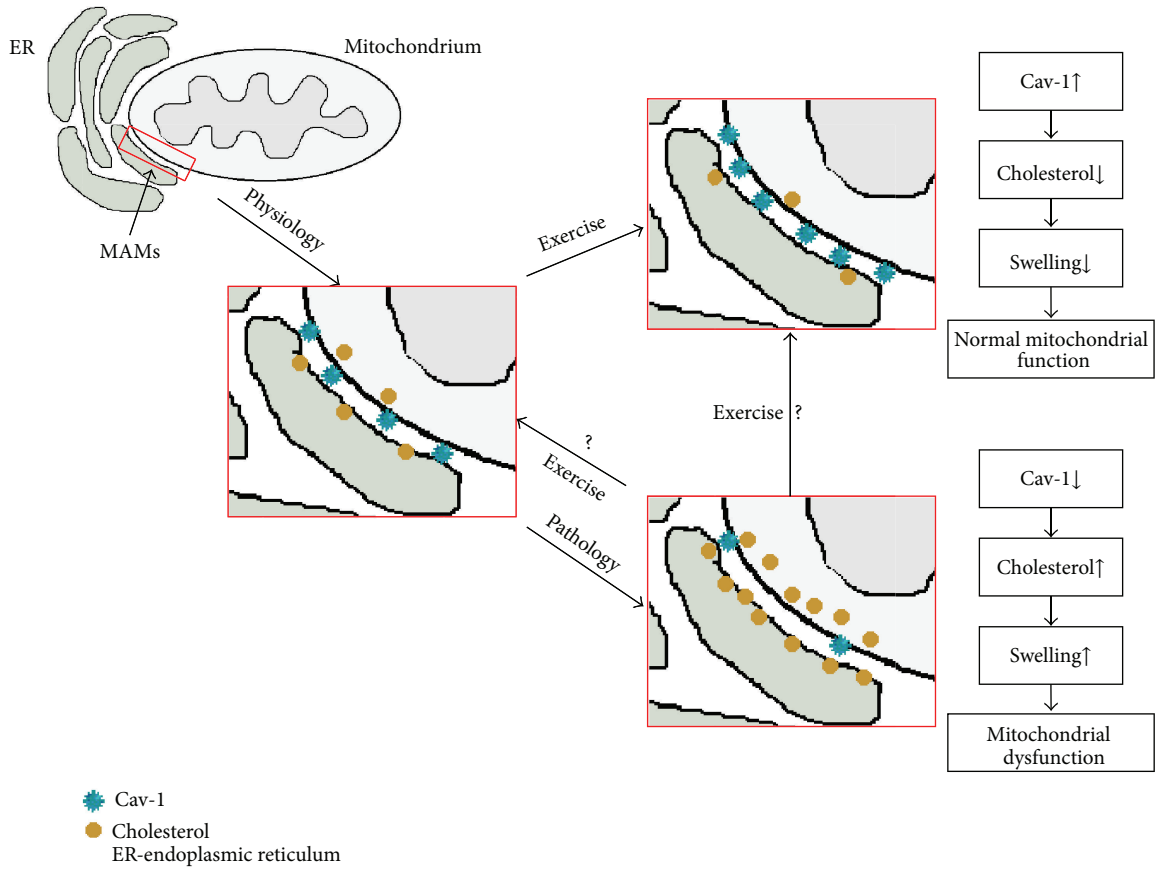

Figure 5: The Cav-1-dependent hypothesis of mitoprotection under exercise conditions. Under physiological conditions: appropriate levels of Cav-1 and cholesterol concentrations are presented in the mitochondria of contractile tissues. Under pathological conditions in crude mitochondria, decreased Cav-1, increased cholesterol concentrations, and mitochondrial swelling are observed. Exercise induces the opposite effects; for example, increased Cav-1 levels and decreased cholesterol levels in mitochondria lead to the inhibition of mitochondria swelling.

cells. These observations have been supported by Chen et al. [30], who reported that Cav-1 is a modifier of the hepatic mitochondrial respiratory chain function, antioxidant enzyme defence system, and mitochondrial biogenesis under hypercholesterolemia-induced oxidative stress.

4.2. The Effect of Changes in Caveolin and Cholesterol Levels on Cellular and Mitochondrial Oxidative Stress and Mitochondrial Swelling. There is an abundance of data which indicate that alternations in caveolin and cholesterol levels in the cell lead to oxidative stress. It was shown that elevated mitochondrial cholesterol levels lead to reduced 2-oxoglutarate transport and an impaired glutathione (GSH) import into mitochondria [15, 31–38]. The reduction of GSH levels is associated with increased oxidative stress [15], which is a key element that initiates mitochondrial swelling [39]. Recently, we demonstrated that the reduction in the mitochondrial cholesterol pool from exercised hearts was not associated with oxidative stress in mitochondria, despite the p66Shc phosphorylation; additionally, there was no induction of cellular apoptosis [1]. The data presented here also support this observation. Prolonged swimming was able to induce oxidative stress; additionally, there was a decrease in the ability to inhibit the mitochondrial swelling in rat livers only. On the contrary, there was no oxidative stress in skeletal muscle mitochondria, where the Cav-1 concentration was higher and the cholesterol level was decreased. Moreover, these organelles were more resistant to the calcium-induced swelling.

Studies in the changes in Cav-1 and cholesterol concentrations in mitochondria seem to be important for understanding the physiological and pathological mechanisms that occur in mitochondria in living organism.

The mechanism behind chronic exercise on the above phenomena remains to be resolved. Based on our previously published results and the results of others [1, 2, 15, 27, 39], it can be suggested that the Cav-1-dependent hypothesis of mitoprotection is under the influence of exercising conditions (Figure 5). Under physiological conditions, appropriate levels of Cav-1 and cholesterol concentrations are present in the mitochondria of contractile tissues. However, in pathological situations, such as ischaemia/reperfusion, decreased Cav-1 and increased cholesterol concentrations in mitochondria are observed. These changes may be associated with remodelling of the mitochondrial raft-like microdomains, mitochondrial swelling, and its respective dysfunction. Exercise induces the opposite effects: for example, increased Cav-1 levels and decreased cholesterol levels lead to the inhibition of

mitochondrial swelling. The mitoprotective effect of exercise is possible only when oxidative stress does not occur in the mitochondria.

The presented data indicate exercise-induced modification in the mitochondrial cholesterol content specific to contractile tissue, which may have resulted from various response to oxidative stress. Moreover, the possible role of Cav-1 on mitochondrial cholesterol depletion following exercise may be fundamental for understanding the mitoprotective mechanism during stressful conditions. Further investigations are required to clarify the role of cholesterol and Cav-1 changes in mitochondria of different tissues following exercise and to explain whether exercise might reverse the mitochondria from a pathological to physiological state via a Cav-1-dependent mechanism.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Rosita Gabbianelli and Wieslaw Ziolkowski share senior coauthorship.

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References

[1] W. Ziolkowski, D. J. Flis, M. Halon et al., "Prolonged swimming promotes cellular oxidative stress and p66Shc phosphorylation, but does not induce oxidative stress in mitochondria in the rat heart," *Free Radical Research*, vol. 49, no. 1, pp. 7–16, 2015.

[2] W. Ziolkowski, S. D. Vadhana M, J. J. Kaczor et al., "Exercise-induced heart mitochondrial cholesterol depletion influences the inhibition of mitochondrial swelling," *Experimental Physiology*, vol. 98, no. 10, pp. 1457–1468, 2013.

[3] A. L. Lehninger, "Water uptake and extrusion by mitochondria in relation to oxidative phosphorylation," *Physiological Reviews*, vol. 42, pp. 467–517, 1962.

[4] K. S. McCommis, A. M. McGee, M. Harold Laughlin, D. K. Bowles, and C. P. Baines, "Hypercholesterolemia increases mitochondrial oxidative stress and enhances the MPT response in the porcine myocardium: Beneficial effects of chronic exercise," *The American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 301, no. 5, pp. R1250–R1258, 2011.

[5] Y. E. Kushnareva and P. M. Sokolove, "Prooxidants open both the mitochondrial permeability transition pore and a low-conductance channel in the inner mitochondrial membrane," *Archives of Biochemistry and Biophysics*, vol. 376, no. 2, pp. 377–388, 2000.

[6] P. Bernardi and A. Rasola, "Calcium and cell death: the mitochondrial connection," *Sub-Cellular Biochemistry*, vol. 45, pp. 481–506, 2007.

[7] R. G. Parton and K. Simons, "The multiple faces of caveolae," *Nature Reviews Molecular Cell Biology*, vol. 8, no. 3, pp. 185–194, 2007.

[8] A. Pol, R. Luetterforst, M. Lindsay, S. Heino, E. Ikonen, and R. G. Parton, "A caveolin dominant negative mutant associates with lipid bodies and induces intracellular cholesterol imbalance," *The Journal of Cell Biology*, vol. 152, no. 5, pp. 1057–1070, 2001.

[9] A. Pol, S. Martin, M. A. Fernández et al., "Cholesterol and fatty acids regulate dynamic caveolin trafficking through the golgi complex and between the cell surface and lipid bodies," *Molecular Biology of the Cell*, vol. 16, no. 4, pp. 2091–2105, 2005.

[10] T. M. Williams and M. P. Lisanti, "The caveolin proteins," *Genome Biology*, vol. 5, no. 3, article 214, 2004.

[11] D. Chen and G. Che, "Value of caveolin-1 in cancer progression and prognosis: emphasis on cancer-associated fibroblasts, human cancer cells and mechanism of caveolin-1 expression (Review)," *Oncology Letters*, vol. 8, no. 4, pp. 1409–1421, 2014.

[12] Y. S. Oh, H. J. Kim, S. J. Ryu et al., "Exercise type and muscle fiber specific induction of caveolin-1 expression for insulin sensitivity of skeletal muscle," *Experimental & Molecular Medicine*, vol. 39, no. 3, pp. 395–401, 2007.

[13] M. S. Mohammadi, D. Thabut, D. Cazals-Hatem et al., "Possible mechanisms involved in the discrepancy of hepatic and aortic endothelial nitric oxide synthases during the development of cirrhosis in rats," *Liver International*, vol. 29, no. 5, pp. 692–700, 2009.

[14] J.-W. Kang and S.-M. Lee, "Impaired expression of caveolin-1 contributes to hepatic ischemia and reperfusion injury," *Biochemical and Biophysical Research Communications*, vol. 450, no. 4, pp. 1351–1357, 2014.

[15] M. Bosch, M. Marí, A. Herms et al., "Caveolin-1 deficiency causes cholesterol-dependent mitochondrial dysfunction and apoptotic susceptibility," *Current Biology*, vol. 21, no. 8, pp. 681–686, 2011.

[16] W. Keatisuwan, M. Kinjo, and T. Koyama, "Changes in phospholipid constituents in mitochondrial membranes after long lasting exercise in rat heart," *Life Sciences*, vol. 48, no. 22, pp. 2173–2181, 1991.

[17] K. M. Broekemeier, P. C. Schmid, H. H. O. Schmid, and D. R. Pfeiffer, "Effects of phospholipase A_2 inhibitors on ruthenium red-induced Ca^{2+} release from mitochondria," *The Journal of Biological Chemistry*, vol. 260, no. 1, pp. 105–113, 1985.

[18] E. Fontaine, O. Eriksson, F. Ichas, and P. Bernardi, "Regulation of the permeability transition pore in skeletal muscle mitochondria. Modulation by electron flow through the respiratory chain complex I," *The Journal of Biological Chemistry*, vol. 273, no. 20, pp. 12662–12668, 1998.

[19] E. D. Crouser, M. E. Gadd, M. W. Julian et al., "Quantitation of cytochrome c release from rat liver mitochondria," *Analytical Biochemistry*, vol. 317, no. 1, pp. 67–75, 2003.

[20] K. Csukly, A. Ascah, J. Matas, P. F. Gardiner, E. Fontaine, and Y. Burelle, "Muscle denervation promotes opening of the permeability transition pore and increases the expression of cyclophilin D," *The Journal of Physiology*, vol. 574, part 1, pp. 319–327, 2006.

[21] M. S. D. Vadhana, M. Carloni, C. Nasuti, D. Fedeli, and R. Gabbianelli, "Early life permethrin insecticide treatment leads to heart damage in adult rats," *Experimental Gerontology*, vol. 46, no. 9, pp. 731–738, 2011.

[22] R. L. Levine, D. Garland, C. N. Oliver et al., "Determination of carbonyl content in oxidatively modified proteins," *Methods in Enzymology*, vol. 186, pp. 464–478, 1990.

[23] V. Misík, K. Ondrias, and P. Balgavý, "Effect of lipid peroxidation on molecular arrangement of phospholipids in liposomes prepared from egg yolk phosphatidylcholine or total rat brain lipids. A 31P NMR study," *General Physiology and Biophysics*, vol. 11, no. 4, pp. 317–325, 1992.

[24] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *The Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.

[25] S. Park, Y. Hong, Y. Lee, J. Won, K. T. Chang, and Y. Hong, "Differential expression of caveolins and myosin heavy chains in response to forced exercise in rats," *Laboratory Animal Research*, vol. 28, no. 1, pp. 1–9, 2012.

[26] S. Park, J. Shin, Y. Hong et al., "Forced exercise enhances functional recovery after focal cerebral ischemia in spontaneously hypertensive rats," *Brain Sciences*, vol. 2, no. 4, pp. 483–503, 2012.

[27] W. Ziolkowski, M. Szkatula, A. Nurczyk et al., "Methyl-beta-cyclodextrin induces mitochondrial cholesterol depletion and alters the mitochondrial structure and bioenergetics," *FEBS Letters*, vol. 584, no. 22, pp. 4606–4610, 2010.

[28] L. A. Martin, B. E. Kennedy, and B. Karten, "Mitochondrial cholesterol: mechanisms of import and effects on mitochondrial function," *Journal of Bioenergetics and Biomembranes*, 2014.

[29] E. Ikonen, "Cellular cholesterol trafficking and compartmentalization," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 2, pp. 125–138, 2008.

[30] Y.-H. Chen, W.-W. Lin, C.-S. Liu, L.-S. Hsu, Y.-M. Lin, and S.-L. Su, "Caveolin-1 provides palliation for adverse hepatic reactions in hypercholesterolemic rabbits," *PLoS ONE*, vol. 9, no. 1, Article ID e71862, 2014.

[31] F. Caballero, A. Fernández, A. M. De Lacy, J. C. Fernández-Checa, J. Caballería, and C. García-Ruiz, "Enhanced free cholesterol, SREBP-2 and StAR expression in human NASH," *Journal of Hepatology*, vol. 50, no. 4, pp. 789–796, 2009.

[32] E. Barbero-Camps, A. Fernández, A. Baulies, L. Martinez, J. C. Fernández-Checa, and A. Colell, "Endoplasmic reticulum stress mediates amyloid beta neurotoxicity via mitochondrial cholesterol trafficking," *American Journal of Pathology*, vol. 184, no. 7, pp. 2066–2081, 2014.

[33] A. Fernandez, N. Matias, R. Fucho et al., "ASMase is required for chronic alcohol induced hepatic endoplasmic reticulum stress and mitochondrial cholesterol loading," *Journal of Hepatology*, vol. 59, no. 4, pp. 805–813, 2013.

[34] J. M. Lluis, A. Colell, C. García-Ruiz, N. Kaplowitz, and J. C. Fernández-Checa, "Acetaldehyde impairs mitochondrial glutathione transport in HepG2 cells through endoplasmic reticulum stress," *Gastroenterology*, vol. 124, no. 3, pp. 708–724, 2003.

[35] A. Colell, C. García-Ruiz, A. Morales et al., "Transport of reduced glutathione in hepatic mitochondria and mitoplasts from ethanol-treated rats: effect of membrane physical properties and S-adenosyl-L-methionine," *Hepatology*, vol. 26, no. 3, pp. 699–708, 1997.

[36] M. Marí, F. Caballero, A. Colell et al., "Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis," *Cell Metabolism*, vol. 4, no. 3, pp. 185–198, 2006.

[37] E. Barbero-Camps, A. Fernández, L. Martínez, J. C. Fernández-Checa, and A. Colell, "APP/PS1 mice overexpressing SREBP-2 exhibit combined Aβ accumulation and tau pathology underlying Alzheimer's disease," *Human Molecular Genetics*, vol. 22, no. 17, Article ID ddt201, pp. 3460–3476, 2013.

[38] L. Llacuna, A. Fernández, C. V. Montfort et al., "Targeting cholesterol at different levels in the mevalonate pathway protects fatty liver against ischemia-reperfusion injury," *Journal of Hepatology*, vol. 54, no. 5, pp. 1002–1010, 2011.

[39] V. P. Skulachev, "Mitochondria in the programmed death phenomena; a principle of biology: 'It is better to die than to be wrong'," *IUBMB Life*, vol. 49, no. 5, pp. 365–373, 2000.

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Research Article

Acute Exercise-Induced Mitochondrial Stress Triggers an Inflammatory Response in the Myocardium via NLRP3 Inflammasome Activation with Mitophagy

Haiying Li,[1,2,3] Weiguo Miao,[2] Jingfen Ma,[2] Zhen Xv,[2] Hai Bo,[2,3] Jianyu Li,[3] Yong Zhang,[2] and Li Li Ji[2,4]

[1] *Tianjin Institute of Health and Environmental Medicine, Tianjin 300050, China*
[2] *Tianjin Key Laboratory of Exercise Physiology and Sports Medicine and Department of Health and Exercise Science, Tianjin University of Sport, Tianjin 300381, China*
[3] *Logistics College of People's Armed Police Forces, Tianjin 300162, China*
[4] *Laboratory of Physiological Hygiene and Exercise Science, School of Kinesiology, University of Minnesota, Minneapolis, MN 55455, USA*

Correspondence should be addressed to Yong Zhang; yzhang@tjus.edu.cn and Li Li Ji; llji@umn.edu

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Increasing evidence has indicated that acute strenuous exercise can induce a range of adverse reactions including oxidative stress and tissue inflammation. However, little is currently known regarding the mechanisms that underlie the regulation of the inflammatory response in the myocardium during acute heavy exercise. This study evaluated the mitochondrial function, NLRP3 inflammasome activation, and mitochondrial autophagy-related proteins to investigate the regulation and mechanism of mitochondrial stress regarding the inflammatory response of the rat myocardium during acute heavy exercise. The results indicated that the mitochondrial function of the myocardium was adaptively regulated to meet the challenge of stress during acute exercise. The exercise-induced mitochondrial stress also enhanced ROS generation and triggered an inflammatory reaction via the NLRP3 inflammasome activation. Moreover, the mitochondrial autophagy-related proteins including Beclin1, LC3, and Bnip3 were all significantly upregulated during acute exercise, which suggests that mitophagy was stimulated in response to the oxidative stress and inflammatory response in the myocardium. Taken together, our data suggest that, during acute exercise, mitochondrial stress triggers the rat myocardial inflammatory response via NLRP3 inflammasome activation and activates mitophagy to minimize myocardial injury.

1. Introduction

Growing evidence suggests that the exercises of varying intensities have distinct effects on the body. Numerous reports have revealed that moderate physical activity enhances the function of various organs and tissues [1]. In contrast, an acute bout of heavy exercise can induce a range of adverse effects including inflammatory response and oxidative stress. Many investigators found that inflammatory cytokines were involved in activating catabolic pathways during an acute bout of exhausting exercise [2]. Although the majority of studies have been conducted in the skeletal muscle, there is evidence that heart can also be subjected to oxidative stress during strenuous physical work under the influence of blood-borne stress hormones (adrenalin and glucocorticoids), proinflammatory cytokines, and locally produced prooxidants [3]. However, the detailed regulatory mechanism involved in the inflammatory response of the myocardium during acute exercise remains unclear.

Mitochondria, the primary energy-producing organelles, play a central role in cellular survival and death. However, mitochondria have recently been found to function as an important source of damage-associated molecular patterns (DAMPs) such as mtDNA, cytochrome C, and reactive

oxygen species (ROS) in inflammatory responses of the myocardium [4, 5]. It has been demonstrated that dysfunctional mitochondria can release DAMPs and enhance inflammatory response, for which activation of the NLRP3 inflammasome is required [6]. The NLRP3 inflammasome, a large multiprotein complex, plays a key regulatory role in the pathogenic processes of the inflammation by modulating its downstream target, IL-1β [7]. The NLRP3 inflammasome is activated by a variety of danger signals integrated from the mitochondria, including infection, metabolic dysfunction, and oxidative stress [8]. Thus, we speculate that mitochondrial stress would trigger NLRP3-induced inflammatory response in the myocardium during acute exercise.

Autophagy is a "self-eating" process that degrades cellular components. Similarly, mitophagy is a catabolic process involved in the removal of dysfunctional mitochondria and a key player in the negative regulation of the NLRP3 inflammasome [9]. Previous evidence has shown that mitophagy is an essential process for maintaining normal cardiac function during stress via the removal of damaged or misfolded mitochondrial proteins [10]. It also serves as a protective response activated by drugs against cardiac injury. For example, simvastatin has been proven to trigger Parkin-dependent mitophagy thereby conferring cardioprotection [11]. Thus, we speculate that an acute bout of exercise may activate mitophagy as a protective response to cardiac inflammatory reactions.

Overall, the current study had the following aims: (1) to investigate the effects of mitochondrial stress on the inflammatory responses of the myocardium during various durations of acute exercise and the subsequent recovery period and (2) to examine the role of NLRP3 inflammasome in the inflammatory responses and the regulation of mitophagy to acute exercise in the myocardium.

2. Methods

2.1. Animals and Groups. Male Sprague-Dawley rats (age 8 weeks) were housed in a temperature-controlled (21-22°C) room. The Institutional Review Board of the Tianjin University of Sport approved all experiments under the guidelines of the Chinese Academy of Sciences. The rats were randomly divided into eight groups ($n = 8$ for each group): the resting control group (RC), the 45 min exercise group (E-45), the 90 min exercise group (E-90), the 120 min exercise group (E-120), the 12 h postexercise recovery group (PE-12), the 24 h postexercise recovery group (PE-24), the 36 h postexercise recovery group (PE-36), and the 48 h postexercise recovery group (PE-48).

2.2. Acute Exercise Model. According to Bedford et al.'s study [12], the rats were subjected to an acute bout of incremental treadmill running for various durations and the maximum oxygen consumption (VO_2max) of rats was calculated on a body mass basis. All groups started running on a motor-driven treadmill for 15 min at 8.2 m/min, 0° grade (~53% VO_2max), followed by 15 min at 15 m/min, 5° grade (~64% VO_2max). Thereafter, three groups of rats ran at 19.3 m/min, 10° grade (~76% VO_2max) continuously for 15, 60, or 90 min and were sacrificed immediately by injection of pentobarbital sodium. Four additional groups of rats performed the exercise protocols described above and were killed at 12, 24, 36, or 48 h after running for a total of 120 min.

2.3. HE Staining. The left ventricles of rats were isolated and the paraffin-embedded sections were prepared, dewaxed, and then incubated in the solution of hematoxylin for 5 min, followed by differentiation with acid alcohol. The nuclei were subsequently stained with ammonia water. Next, the slides were incubated in the eosin solution for 2 min. After dehydration, clearing, and mounting, the sections were observed using a light microscope (Nikon E200, Tokyo, Japan).

2.4. Heart Mitochondrial Isolation. The left ventricular mitochondria of rats were prepared using differential centrifugation according to established protocols [13]. Briefly, the minced blood-free tissue was homogenized in the buffer solution containing 0.25 M sucrose, 3.0 mM Hepes, and 0.5 mM EDTA (pH 7.4). The homogenate was centrifuged at 800 ×g for 10 min, and the resulting supernatant was centrifuged at 10,000 ×g for 10 min. The pellets were gently resuspended and centrifuged again at 10,000 ×g for 10 min, and the final pellets were suspended in 1 mL of the isolation medium. The mitochondrial protein content was detected using the Bradford assay.

2.5. Mitochondrial Respiration. Mitochondrial respiratory function was measured using a high-resolution respirometer, Oxygraph-2k (Oroboros Co., Austria). The reactions were conducted at 37°C in a thermostated and magnetically stirred chamber containing 130 mM KCl, 10.0 mM Hepes, 1 mM EDTA, 2.5 mM KH_2PO_4, and 1 mg/mL BSA (pH 7.4) with 0.3 mg mitochondrial protein. After a 10 min equilibration period, mitochondrial respiration was initiated by adding 2 M pyruvic acid sodium and 0.8 M malate. After a stable state 2 respiration (ST2) was established, a state 3 respiration (ST3) was initiated by the addition of 0.5 M ADP. After all of the added ADP had been phosphorylated to ATP, the respiratory rate returned to state 4. The respiratory control ratio (RCR) was calculated as the ratio of the respiratory rate in state 3 to that in state 4.

2.6. Mitochondrial Membrane Potential and ATP Synthase Activity. The isolated mitochondrial membrane potential ($\Delta\psi$) was measured by monitoring the fluorescence spectrum of JC-1 [14]. The experiments were performed at 37°C in an incubation medium containing 1.5 mL JC-1 staining solution, 2 M pyruvic acid sodium, and 0.8 M malate with 0.3 mg mitochondrial protein. ATP synthase activity was determined using a bioluminescence technique [15]. The mitochondrial suspensions were added to a cuvette containing 40 μM luciferase (Sigma, MO, USA), 0.25 M sucrose, 3.0 mM Hepes, 0.5 mM EDTA, 2 M pyruvate, and 0.8 M malate. After a background bioluminescence was established for correction, 0.5 μM ADP was added to initiate the reaction. ATP production was monitored at 37°C using a BioOrbit 20/20n

luminometer (Turku, Finland) and expressed as nanomoles per minute per mg protein.

2.7. Mitochondrial ROS Production. The mitochondrial ROS generation rate was monitored using a dichlorofluorescein diacetate (H2-DCFDA) probe [16]. Briefly, the H2-DCFDA stock solution was dissolved in 1.25 mM methanol in a dark room at 0°C. To initiate the experiment, 0.3 mg mitochondrial protein was added to a quartz cuvette containing 2.5 mL of 0.1 M phosphate buffer (pH 7.4) and 2 μL of 2.5 mM H2-DCFDA. The assay mixture was incubated at 37°C for 15 min. DCF formation was determined fluorometrically at an excitation wavelength of 499 nm and an emission wavelength of 521 nm at 37°C for 2 min, using a Cary Eclipse fluorescence spectrophotometer (Varian, CA, USA). The units were expressed as a picomole of DCF formed per minute per mg of protein.

2.8. Mitochondrial MnSOD Activity and MDA Content. SOD activities were measured using the method [17], where one unit of SOD activity was defined as the amount of enzyme that causes a 50% inhibition of epinephrine autoxidation to adrenochrome. To determine the MnSOD activity, 1 mM KCN^- was added to the reaction mixture to inhibit the activity of CuZnSOD. The content of MDA was measured using the thiobarbituric acid method. In brief, the MDA in mitochondrial samples reacted with thiobarbituric acid and a pink-colored product produced in the reaction. For detecting the contents of MDA, the reaction products were quantified as the absorbance at 532 nm according to the manufacturer's instructions.

2.9. Western Blotting Analysis. The left ventricular tissue samples of rats were freshly homogenized in the lysis buffer containing protease inhibitor PMSF (phenylmethanesulfonyl fluoride). The homogenates were solubilized using end-over-end mixing for 60 min at 4°C followed by centrifugation at 15,000 ×g for 20 min at 4°C. The total protein concentration was determined using the Bradford assay. The whole-cell proteins solubilized in the sample buffer were separated using SDS-PAGE and transferred to PVDF membranes (Millipore, MA, USA). The membranes were blocked and exposed to primary rabbit polyclonal antibodies (anti-Bnip3, 1 : 500; anti-Beclin1, 1 : 1000; anti-LC3, 1 : 500; anti-IL-1β, 1 : 1000; and anti-β-tubulin, 1 : 3000) (Abcam, Cambridge, UK) and primary goat polyclonal antibody (anti-NLRP3, 1 : 500) (Abcam, Cambridge, UK) overnight at 4°C. The membranes were washed and incubated with the HRP-conjugated anti-rabbit IgG (1 : 10000) and the HRP-conjugated anti-goat IgG (1 : 10000) (CST, Boston, USA) for 60 minutes. The data were analyzed using Quantity One software (Bio-Rad, CA, USA) to obtain the optical density ratio of target proteins relative to β-tubulin.

2.10. Statistical Analyses. The data were presented as the mean ± SEM and analyzed using a one-way ANOVA followed by Dunnett's many-to-one test. Statistical Package for the Social Sciences (SPSS Inc., version 15.0) was used for all analyses. The significance level was set at $p < 0.05$.

3. Results

3.1. Pathological Assessment. In this study, no apparent changes in the morphology or structure of the myocardial tissues were found during acute exercise and during the recovery; however, myocardial tissues were found to display more dilatation and congestion of capillaries and inflammatory cell infiltration in the E-45, E-90, and E-120 groups, compared with those of the RC group. Moreover, characteristic inflammatory responses in the rat myocardium were also observed during the early stage of recovery in the PE-12 and PE-24 groups (Figure 1).

3.2. Mitochondrial Respiratory Function in the Myocardium. Compared with that of the RC group, mitochondrial ST3 respiration rate was elevated by 68.9% ($p < 0.01$) in the E-45 group but decreased by 40.6% ($p < 0.05$) in the E-120 group. During the recovery, ST3 increased by 48.9% again ($p < 0.05$) in the PE-24 group before returning to the RC level in the PE-36 and PE-48 groups (Figure 2(a)). ST4 respiration rate was increased by 23.1% and 24.3% ($p < 0.05$) in both E-45 and E-120 groups and then returned to the RC level in all PE groups (Figure 2(b)). Similar to ST3 respiration rate, RCR was increased by 34.3% in the PE-45 group ($p < 0.05$), decreased by 50.3% in the E-120 group ($p < 0.01$), and rebounded by 32.6% in the PE-24 group ($p < 0.05$) (Figure 2(c)).

3.3. Mitochondrial Membrane Potential and ATP Synthase Activity. Compared with that of the RC group, mitochondrial membrane potential was increased by 32.2% ($p < 0.05$) in the E-45 group but decreased by 29.4% ($p < 0.05$) in the E-120 group; it returned to the RC level in all PE groups (Figure 3(a)). Mitochondrial ATP synthase activity was elevated by 66.6% ($p < 0.01$) in the E-45 group but decreased by 45.9% ($p < 0.05$) in the E-120 group. During the recovery, ATP synthase activity increased by 50.0% again ($p < 0.05$) in the PE-24 group before returning to the RC level in the PE-36 and PE-48 groups (Figure 3(b)).

3.4. Mitochondrial Prooxidants and Antioxidants. Compared with that of the RC group, the mitochondrial ROS generation rate was increased by 37.5%, 82.6%, and 90.4% in the E-45 ($p < 0.05$), E-90 ($p < 0.01$), and E-120 ($p < 0.01$) groups during acute exercise and also enhanced by 56.0% ($p < 0.01$) during the early stage of recovery (PE-12 group). It then returned to the RC level in the PE-24, PE-36, and PE-48 groups (Figure 4(a)). Mitochondrial MDA content was increased by 19.5%, 22.8%, and 23.6% in the E-45 ($p < 0.05$), E-90 ($p < 0.01$), and E-120 ($p < 0.01$) groups during acute exercise and also enhanced by 17.7% ($p < 0.05$) during the early stage of recovery (PE-12 group). It then returned to the RC level in the PE-24, PE-36, and PE-48 groups (Figure 4(b)). MnSOD activity did not change during the exercise period or the early stage of recovery (PE-12 and PE-24 groups) and then

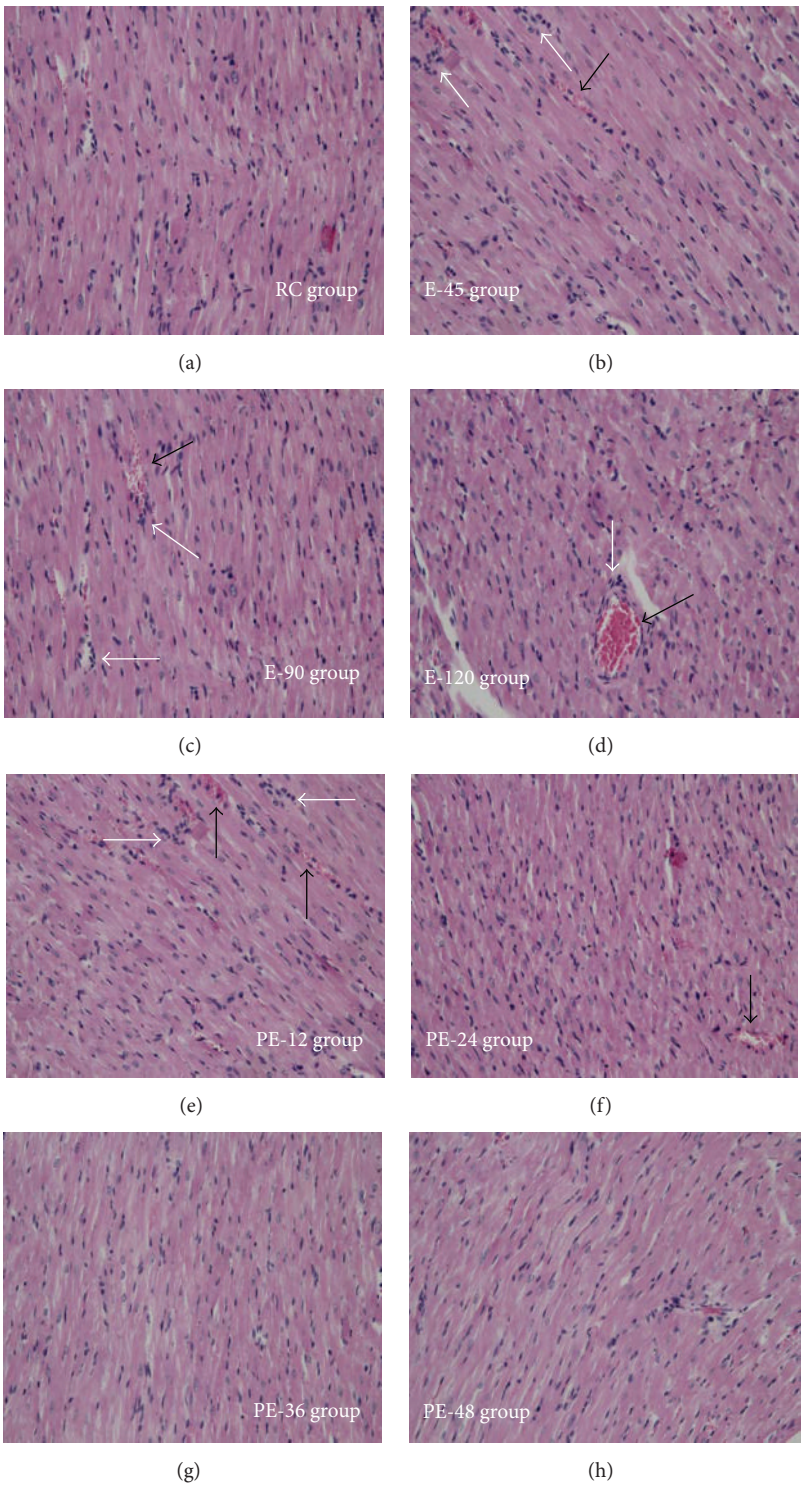

Figure 1: The pathological changes of the rat myocardium during acute exercise were observed using HE staining. Compared with those of the RC group (a), more dilatation and congestion of capillaries (the highlight of the black arrows) and inflammatory cell infiltration (the highlight of the white arrows) were observed in the cardiac tissues during the exercise period ((b), (c), and (d)) and the early stage of recovery ((e) and (f)).

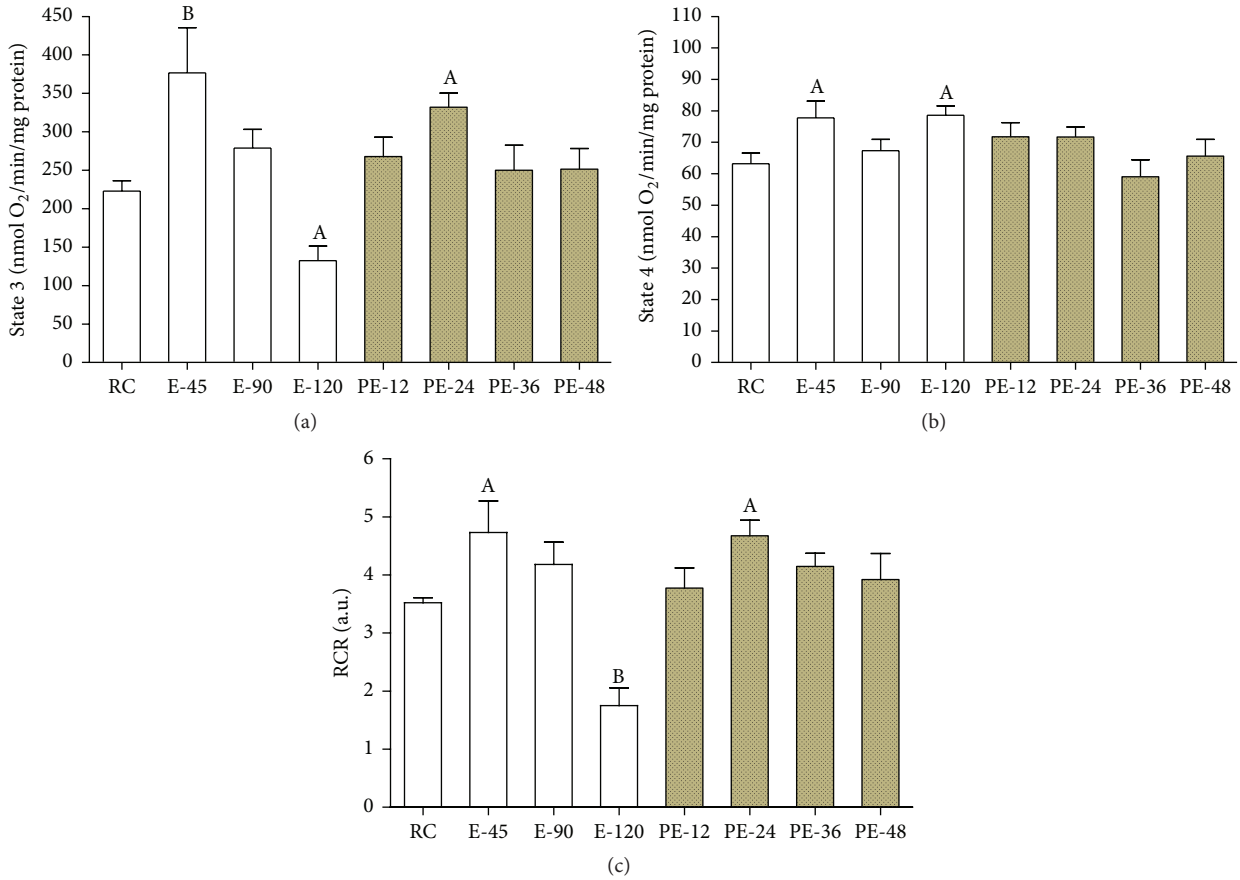

Figure 2: To investigate the mitochondrial respiratory function in the myocardium, mitochondrial ST3, ST4, and RCR were determined. Compared with that of the RC group, mitochondrial ST3 and RCR were both increased during the early stages of both acute exercise and its recovery ((a) and (c)), and ST4 was increased only during acute exercise (b). Each bar represents the mean ± SEM of a treatment group ($n = 8$). $^{A}p < 0.05$ and $^{B}p < 0.01$ versus RC group.

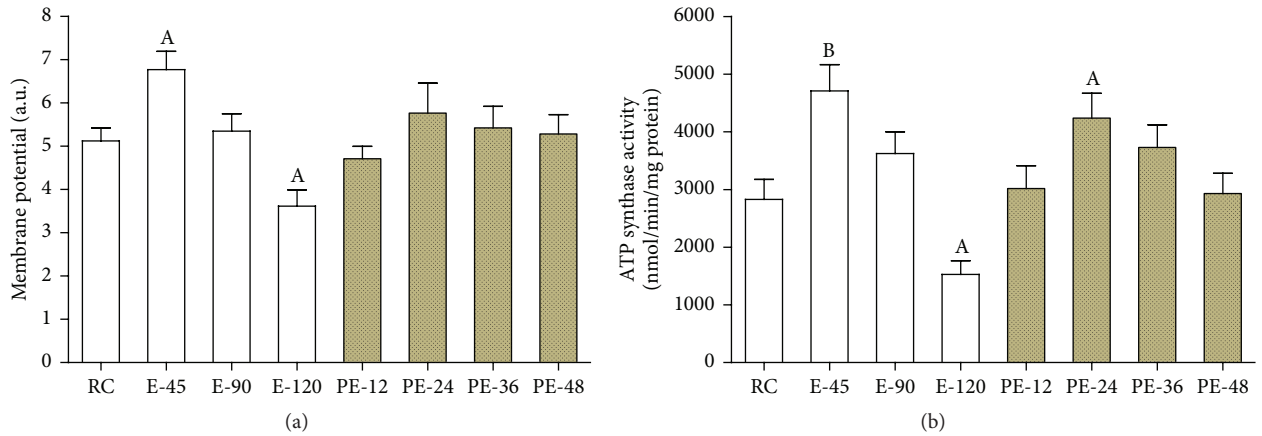

Figure 3: To investigate the mitochondrial stress response in the myocardium, mitochondrial membrane potential and ATP synthase activity were determined. Compared with that of the RC group, mitochondrial membrane potential and ATP synthase activity were both increased during the early stages of acute exercise and/or its recovery ((a) and (b)). Each bar represents the mean ± SEM of a treatment group ($n = 8$). $^{A}p < 0.05$ and $^{B}p < 0.01$ versus RC group.

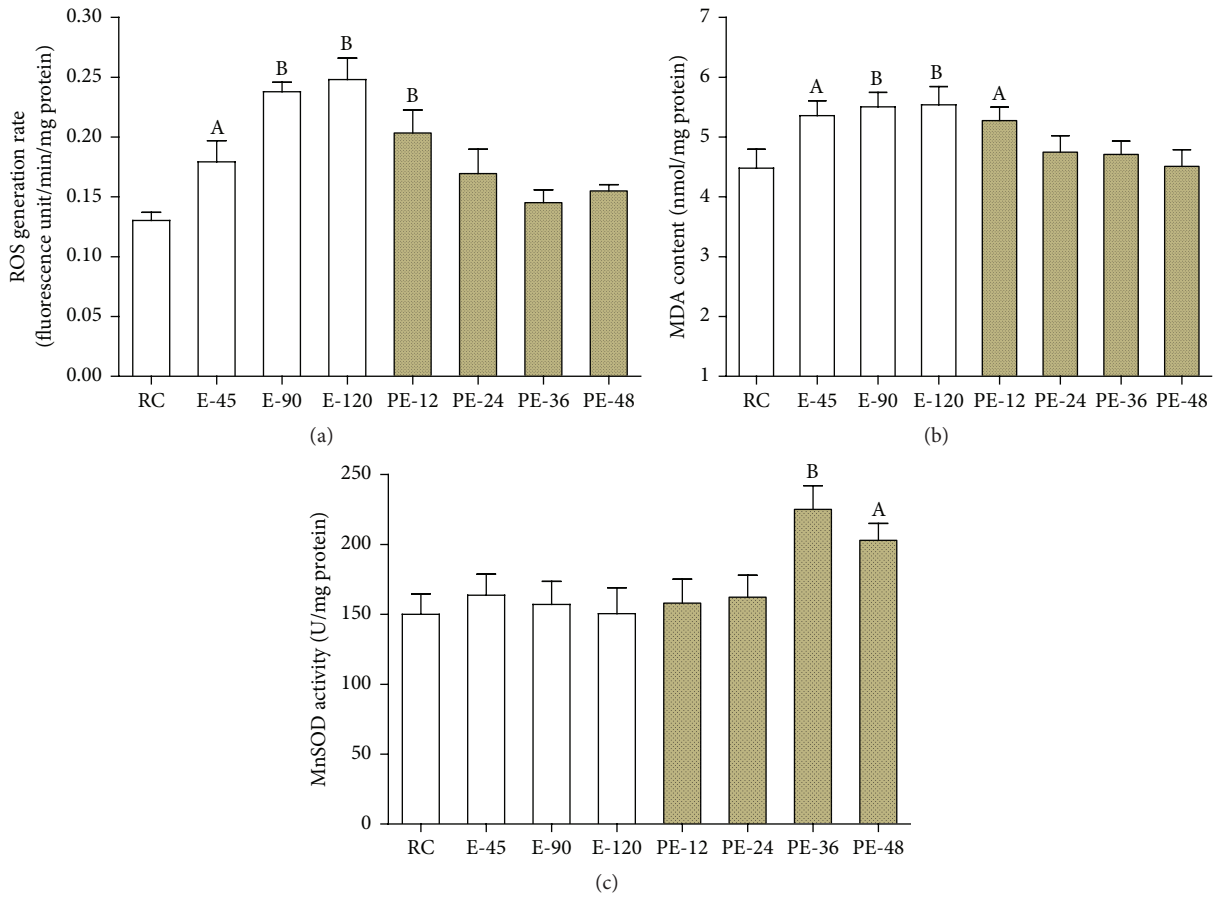

Figure 4: To investigate the mitochondrial oxidative stress response in the myocardium, ROS generation rate, MnSOD activity, and MDA content were measured. Compared with that of the RC group, mitochondrial ROS generation and MDA content were both increased during acute exercise and early recovery ((a) and (b)), but MnSOD activity was not increased until late recovery (c). Each bar represents the mean ± SEM of a treatment group ($n = 8$). $^{A}p < 0.05$ and $^{B}p < 0.01$ versus RC group.

was increased by 50.1% and 35.3% in the PE-36 ($p < 0.01$) and PE-48 ($p < 0.05$) groups (Figure 4(c)).

3.5. The Expressions of NLRP3 and Its Downstream Target IL-1β. NLRP3 protein content was increased by 48.1% in E-45 versus RC ($p < 0.05$), but the change was not significant in E-90 or E-120. During the recovery, NLRP3 level was increased by 63.9% and 51.2% again in PE-12 ($p < 0.01$) and PE-24 ($p < 0.05$) before returning to RC level (Figure 5(a)). IL-1β expression was upregulated by 30.5% in E-45 versus RC ($p < 0.05$), but the change was not significant in E-90 or E-120. During the recovery, IL-1β level was enhanced by 100.0% and 77.0% again in PE-12 and PE-24 ($p < 0.01$) before returning to RC level (Figure 5(b)).

3.6. The Expressions of Mitochondrial Autophagy-Related Proteins. Beclin1 expressions were enhanced by 46.2% in E-45 versus RC ($p < 0.05$), but they returned to the RC level in the E-90 and E-120 groups. During the recovery, Beclin1 level was increased by 43.9% and 88.2% again in the PE-12 ($p < 0.05$) and PE-24 ($p < 0.01$) groups before returning to the RC level (Figure 6(a)). Bnip3 protein content was upregulated by 55.1% in E-45 versus RC ($p < 0.05$), but the change was not significant in E-90 or E-120. During the recovery, Bnip3 level was increased by 73.9% and 52.4% again in the PE-12 ($p < 0.01$) and PE-24 ($p < 0.05$) groups before returning to the RC level (Figure 6(b)). The expressions of total LC3 increased by 65.2% and the ratio of LC3 II to LC3 I increased by 36.7% in the E-45 group ($p < 0.05$) but returned to the RC level in the E-90 and E-120 groups. During the recovery, the expressions of total LC3 increased by 95.5% and 66.5% again in the PE-12 ($p < 0.01$) and PE-24 ($p < 0.05$) groups, and the ratio of LC3 II to LC3 I increased by 48.3% and 40.1% again in the PE-12 ($p < 0.01$) and PE-24 ($p < 0.05$) groups before returning to the RC level (Figures 6(c) and 6(d)).

4. Discussion

Accumulating evidence has shown that moderate exercise can result in an increased resistance to oxidative challenge by maintaining the balance between prooxidants and antioxidants [18]. On the other hand, strenuous exercise (e.g., acute fatiguing exercise) can promote the excessive generation of

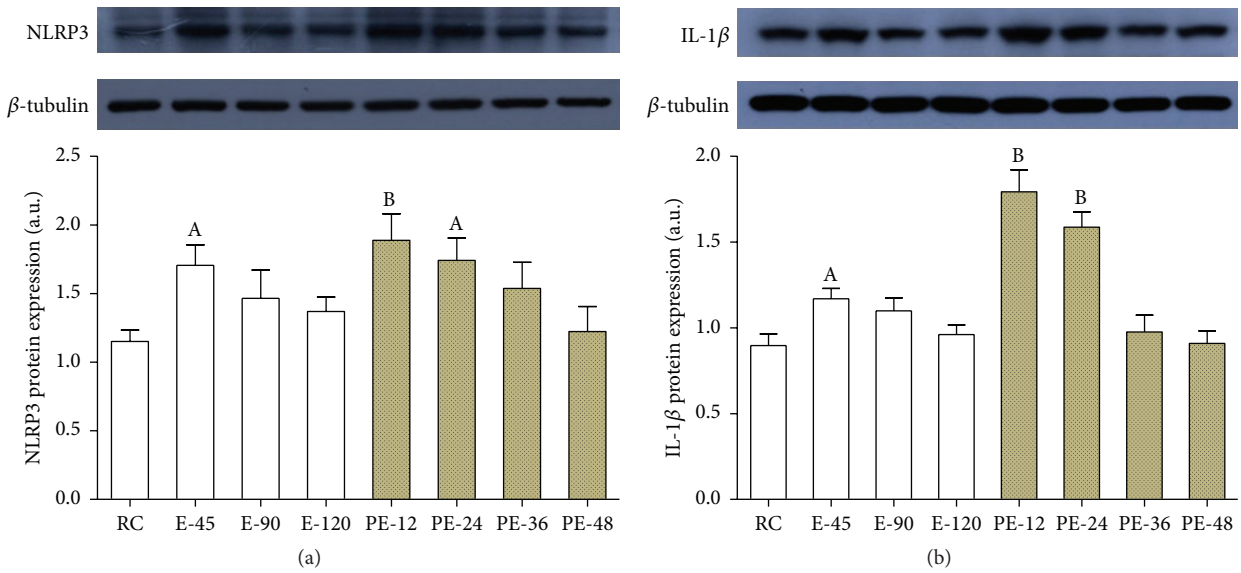

Figure 5: To investigate the inflammatory response in the myocardium, the expressions of NLRP3 and its target IL-1β were determined. The data showed that the expressions of NLRP3 and IL-1β were upregulated in the early stage of both acute exercise and its recovery ((a) and (b)). Each bar represents the mean ± SEM of a treatment group ($n = 8$). $^{A}p < 0.05$ and $^{B}p < 0.01$ versus RC group.

ROS and lead to transient immunosuppressant and inflammatory responses [19].

Oxidative stress and inflammation have been found to be associated with an excess of ROS, which could potentially be a main source of mitochondrial dysfunction. Recent studies have found that the damage of muscle tissue arising from strenuous exercise increases the level of ROS and induces an inflammatory response [20]. Regarding the sources of ROS in exercise, it is generally accepted that the superoxide is mainly produced by mitochondria, phagocytes, and xanthine oxidase (XO) [21]. During exercise, one of the most common forms of ROS generation is due to electron leaks from the mitochondrial electron transport chain (ETC). In this research, we used the DCFH-DA probe to measure the generation of mitochondrial ROS and used the TBARS assay to detect MDA content in mitochondria during acute exercise. We acknowledge the limitations in the above methods as have been reported [22, 23]; however, the DCFH-DA probe and the TBARS assay continue to be used for the assessment of ROS and MDA level [24, 25]. The results of the current study showed that the ROS generation and MDA content in the mitochondria of rat myocardium significantly increased during acute exercise and early recovery; however, the antioxidant enzyme MnSOD in mitochondria was not enhanced until 36 and 48 hours after exercise. NLRP3, an important inflammatory factor, is implicated in various inflammatory responses, and its function primarily depends on a multiprotein complex called the inflammasome. The formation of the NLRP3 inflammasome and the production of its downstream target IL-1β are generally thought to be responsible for the mitochondrial ROS-mediated inflammatory reaction to a variety of stresses [26]. It has been demonstrated that ROS production derived from mitochondria in the myocardium could induce the oligomerization of NLRP3, recruitment of apoptosis-associated speck-like protein containing a CARD domain (ASC), and activation of caspase-1 [27]. The activation of caspase-1 can transform pro-IL-1β to mature IL-1β, leading to enhanced inflammation and mitochondrial dysfunction [28]. Finally, damaged mitochondria may produce greater amount of ROS and form a vicious cycle of inflammatory response [29]. This scenario is consistent with our data showing that the upregulated expressions of NLRP3 and IL-1β took place at the early stage of exercise (E-45), followed by a surge of ROS production. Furthermore, morphological parameters such as the dilatation and congestion of capillaries as well as inflammatory cell infiltration were observed in the rat myocardium, providing further support for the above point of view. These results suggest that NLRP3 inflammasome activation and subsequent inflammatory response might play a key role in explaining the mitochondrial stress response in the myocardium, such as enhanced ST3 respiration and RCR, as well as ATP production in rats exercised for two hours (Figures 2(a), 2(c), and 3(b)).

To investigate the detailed mechanism involved in the regulation of the inflammatory response of the myocardium during acute exercise, mitochondrial functions of the rat myocardium, including the ATP synthase activity, membrane potential, and respiratory function of mitochondria, were determined. ATP generation should be required to supply energy to maintain the dynamics of myocardial contraction during acute heavy exercise. In this study, our data indicated that the activities of mitochondrial ATP synthase were enhanced during the early stages of both acute exercise and its recovery, and this finding was accompanied by increased levels of ROS and the upregulation of inflammatory factors. These effects suggest that the enhanced process of oxidative phosphorylation promoted the consumption of oxygen, induced the overproduction of ROS, and

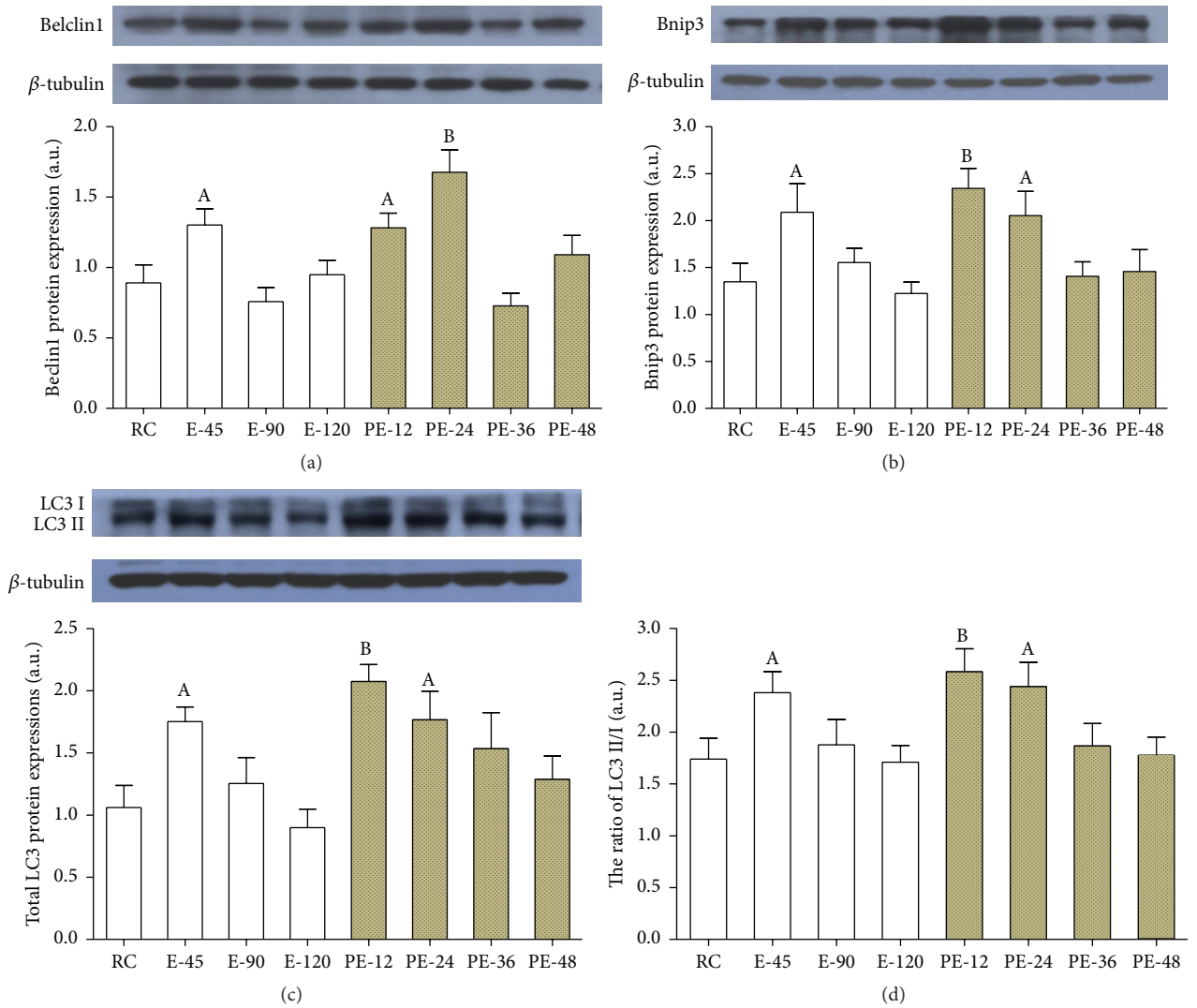

Figure 6: To investigate the mitophagy in the myocardium, the expressions of several mitochondrial autophagy-related proteins were examined. Compared with that of the RC group, the expressions of Beclin1, Bnip3, and total LC3, as well as the ratio of LC3 II to LC3 I, were all increased during the early stages of both acute exercise and its recovery ((a), (b), (c), and (d)). Each bar represents the mean ± SEM of a treatment group ($n = 8$). $^{A}p < 0.05$ and $^{B}p < 0.01$ versus RC group.

stimulated the mitochondrial ROS-mediated inflammatory response.

In addition to increased ATP production, we found that the membrane potential of mitochondria was elevated during the mitochondrial stress response associated with the early stage of acute exercise. Increased mitochondrial membrane potential can promote the formation of ROS, and the levels of ROS were exponentially enhanced at membrane potentials above 140 mV [30]. Thus, we assumed that the elevated membrane potential of mitochondria might precipitate increased ROS generation and the inflammatory response during acute exercise. Mitochondrial RCR, calculated as ST3 divided by ST4, is a critical parameter of mitochondrial respiratory function and decreases in this parameter are involved in the dysfunction of mitochondria in aging and various diseases [31]. However, the data in the current study revealed that mitochondrial RCR was enhanced during the early stages of both acute exercise and its recovery, which might be associated with the mitochondrial stress and increased ROS generation. Given the above findings, we concluded that the mitochondrial function of the myocardium is adaptively regulated to meet the challenge of stress during acute exercise and its recovery period; however, ROS generation and the inflammatory reaction were simultaneously triggered and enhanced via the mitochondrial stress response.

Autophagy, an evolutionary pathway for the catabolism of cellar components, is closely associated with the lysosomal degradation of damaged organelles such as mitochondria [32]. When the autophagic process of selective degradation occurs within the mitochondria, it is known as mitophagy and an important regulated pathway that plays a crucial role in maintaining cellular homeostasis in various

tissues [33]. Increasing evidence suggests that dysfunctional mitophagy is responsible for the deteriorations of human bodies associated with aging and disease [34, 35]. Recent studies have demonstrated that dysregulated mitophagy is involved in the pathogenesis of diabetic cardiomyopathy [36]. The current knowledge indicates that many positive and negative modulators of mitophagy, such as Beclin1, Atg14L, UVRAG, LC3, and Bnip3, tightly regulate the progression of mitophagy. AMP-activated protein kinase (AMPK), an essential energy sensor, regulates the induction of mitophagy via the phosphorylation of Beclin1 [37], whereas LC3 includes Pre-LC3, LC3 I, and LC3 II and plays a key regulatory role in the initiation of mitophagy on the autophagosome membrane [38]. Bnip3, a key regulatory factor for mitophagy, is essential for the formation of homodimers of Bnip3 with LC3 [39, 40].

To investigate the status of mitophagy in the myocardium during acute exercise and its recovery, we examined the expressions of several mitochondrial autophagy-related proteins including Beclin1, LC3, and Bnip3. Our data showed that these mitophagy-related proteins were all significantly upregulated during the early stages of both acute exercise and recovery. Noticeably, the pattern of the changes in these proteins is nearly the same as those for NLRP3 and IL-1β. These results suggest that the induction of mitophagy in the myocardium might be involved in the regulation of inflammatory reactions during the mitochondrial stress response. It has been demonstrated that accumulation of mitochondrial ROS can activate mitophagy and that mitophagy serves as a means for clearance of overproduced ROS [41]. Moreover, the blockade of mitophagy can result in the overgeneration of ROS and induce the activation of NLRP3 inflammasome in turn, which suggests that induced mitophagy mitigates inflammatory response via the negative regulation of ROS generation and NLRP3 inflammasome activation [9]. Thus, we suggest that the increased mitophagy observed in this study was stimulated to minimize myocardial injury via suppressing ROS-induced inflammatory response during acute exercise. Other studies have also revealed that mitophagy can be induced to various harmful stresses and proper induction of autophagy can maintain muscle homeostasis during exercise, which appears to be an adaptive response to protect against injury [42–44]. Additionally, the decreased mitochondrial function in E-120 versus RC might be associated with overstimulation of mitophagy in this study.

5. Conclusion

In summary, we conclude that mitochondrial stress triggers the rat myocardium inflammatory response via activation of the NLRP3 inflammasome and induces mitophagy to minimize myocardial injury during acute exercise (Figure 7).

Conflict of Interests

The authors have no conflict of interests to declare.

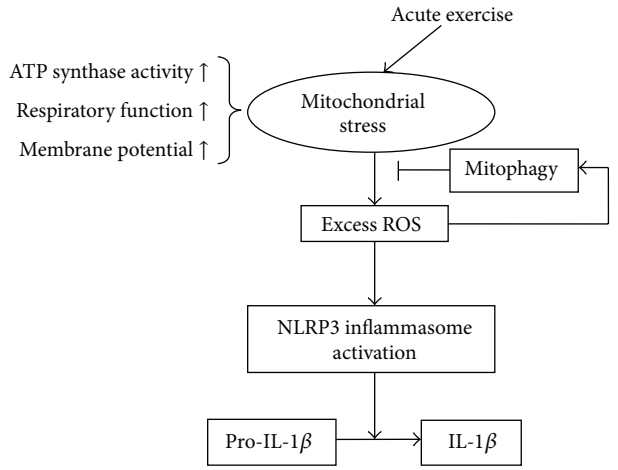

Figure 7: Acute exercise-induced mitochondrial stress triggers an inflammatory response in the myocardium via NLRP3 inflammasome activation with mitophagy.

Acknowledgments

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References

[1] S. K. Powers, J. C. Quindry, and A. N. Kavazis, "Exercise-induced cardioprotection against myocardial ischemia-reperfusion injury," *Free Radical Biology and Medicine*, vol. 44, no. 2, pp. 193–201, 2008.

[2] A. Abbasi, M. Hauth, M. Walter et al., "Exhaustive exercise modifies different gene expression profiles and pathways in LPS-stimulated and un-stimulated whole blood cultures," *Brain, Behavior, and Immunity*, vol. 39, pp. 130–141, 2014.

[3] D. König, O. Neubauer, L. Nics et al., "Biomarkers of exercise-induced myocardial stress in relation to inflammatory and oxidative stress," *Exercise Immunology Review*, vol. 13, pp. 15–36, 2007.

[4] Q. Zhang, M. Raoof, Y. Chen et al., "Circulating mitochondrial DAMPs cause inflammatory responses to injury," *Nature*, vol. 464, no. 7285, pp. 104–107, 2010.

[5] W. Zhao, T. Zhao, Y. Chen, Y. Qu, I. C. Gerling, and Y. Sun, "Modification of oxidative stress on gene expression profiling in the rat infarcted heart," *Molecular and Cellular Biochemistry*, vol. 379, no. 1-2, pp. 243–253, 2013.

[6] S. Yang, C. Xia, S. Li, L. Du, L. Zhang, and R. Zhou, "Defective mitophagy driven by dysregulation of rheb and KIF5B contributes to mitochondrial reactive oxygen species (ROS)-induced nod-like receptor 3 (NLRP3) dependent proinflammatory response and aggravates lipotoxicity," *Redox Biology*, vol. 3, pp. 63–71, 2014.

[7] N. A. Bracey, P. L. Beck, D. A. Muruve et al., "The Nlrp3 inflammasome promotes myocardial dysfunction in structural

cardiomyopathy through interleukin-1β," *Experimental Physiology*, vol. 98, no. 2, pp. 462–472, 2013.

[8] D. V. Krysko, P. Agostinis, O. Krysko et al., "Emerging role of damage-associated molecular patterns derived from mitochondria in inflammation," *Trends in Immunology*, vol. 32, no. 4, pp. 157–164, 2011.

[9] R. Zhou, A. S. Yazdi, P. Menu, and J. Tschopp, "A role for mitochondria in NLRP3 inflammasome activation," *Nature*, vol. 469, no. 7329, pp. 221–225, 2011.

[10] B. C. Hammerling and A. B. Gustafsson, "Mitochondrial quality control in the myocardium: cooperation between protein degradation and mitophagy," *Journal of Molecular and Cellular Cardiology*, vol. 75, pp. 122–130, 2014.

[11] A. M. Andres, G. Hernandez, P. Lee et al., "Mitophagy is required for acute cardioprotection by simvastatin," *Antioxidants and Redox Signaling*, vol. 21, no. 14, pp. 1960–1973, 2014.

[12] T. G. Bedford, C. M. Tipton, N. C. Wilson, R. A. Oppliger, and C. V. Gisolfi, "Maximum oxygen consumption of rats and its changes with various experimental procedures," *Journal of Applied Physiology Respiratory Environmental and Exercise Physiology*, vol. 47, no. 6, pp. 1278–1283, 1979.

[13] L. L. Ji, D. Dillon, and E. Wu, "Myocardial aging: antioxidant enzyme systems and related biochemical properties," *American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 261, no. 2, pp. R386–R392, 1991.

[14] K. Chen, Q. Zhang, J. Wang et al., "Taurine protects transformed rat retinal ganglion cells from hypoxia-induced apoptosis by preventing mitochondrial dysfunction," *Brain Research*, vol. 1279, pp. 131–138, 2009.

[15] X. Chen, D. B. Jennings, and D. M. Medeiros, "Impaired cardiac mitochondrial membrane potential and respiration in copper-deficient rats," *Journal of Bioenergetics and Biomembranes*, vol. 34, no. 5, pp. 397–406, 2002.

[16] H. Bo, N. Jiang, G. D. Ma et al., "Regulation of mitochondrial uncoupling respiration during exercise in rat heart: role of reactive oxygen species (ROS) and uncoupling protein 2," *Free Radical Biology and Medicine*, vol. 44, no. 7, pp. 1373–1381, 2008.

[17] M. Sun and S. Zigman, "An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation," *Analytical Biochemistry*, vol. 90, no. 1, pp. 81–89, 1978.

[18] L. L. Ji and Y. Zhang, "Antioxidant and anti-inflammatory effects of exercise: role of redox signaling," *Free Radical Research*, vol. 48, no. 1, pp. 3–11, 2014.

[19] C.-C. Huang, T.-J. Lin, Y.-F. Lu, C.-C. Chen, C.-Y. Huang, and W.-T. Lin, "Protective effects of L-arginine supplementation against exhaustive exercise-induced oxidative stress in young rat tissues," *The Chinese Journal of Physiology*, vol. 52, no. 5, pp. 306–315, 2009.

[20] W. Aoi, Y. Naito, and T. Yoshikawa, "Role of oxidative stress in impaired insulin signaling associated with exercise-induced muscle damage," *Free Radical Biology and Medicine*, vol. 65, pp. 1265–1272, 2013.

[21] S. Sachdev and K. J. A. Davies, "Production, detection, and adaptive responses to free radicals in exercise," *Free Radical Biology and Medicine*, vol. 44, no. 2, pp. 215–223, 2008.

[22] B. Kalyanaraman, V. Darley-Usmar, K. J. A. Davies et al., "Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations," *Free Radical Biology and Medicine*, vol. 52, no. 1, pp. 1–6, 2012.

[23] H. J. Forman, O. Augusto, R. Brigelius-Flohe et al., "Even free radicals should follow some rules: a guide to free radical research terminology and methodology," *Free Radical Biology and Medicine*, vol. 78, no. 1, pp. 233–235, 2015.

[24] C. S. Xu, Z. F. Wang, X. D. Huang, L. M. Dai, C. J. Cao, and Z. Q. Li, "Involvement of ROS-alpha v beta 3 integrin-FAK/Pyk2 in the inhibitory effect of melatonin on U251 glioma cell migration and invasion under hypoxia," *Journal of Translational Medicine*, vol. 13, article 95, 2015.

[25] I. Layali, E. Tahmasbpour, M. Joulaei, S. G. A. Jorsaraei, and P. Farzanegi, "Total antioxidant capacity and lipid peroxidation in semen of patient with hyperviscosity," *Cell Journal*, vol. 16, no. 4, pp. 554–559, 2015.

[26] M. E. Heid, P. A. Keyel, C. Kamga, S. Shiva, S. C. Watkins, and R. D. Salter, "Mitochondrial reactive oxygen species induces NLRP3-dependent lysosomal damage and inflammasome activation," *Journal of Immunology*, vol. 191, no. 10, pp. 5230–5238, 2013.

[27] M. Takahashi, "Role of the inflammasome in myocardial infarction," *Trends in Cardiovascular Medicine*, vol. 21, no. 2, pp. 37–41, 2011.

[28] M. I. R. Reis, A. do Vale, P. J. B. Pereira, J. E. Azevedo, and N. M. S. dos Santos, "Caspase-1 and IL-1β processing in a teleost fish," *PLoS ONE*, vol. 7, no. 11, Article ID e50450, 2012.

[29] M. J. López-Armada, R. R. Riveiro-Naveira, C. Vaamonde-García, and M. N. Valcárcel-Ares, "Mitochondrial dysfunction and the inflammatory response," *Mitochondrion*, vol. 13, no. 2, pp. 106–118, 2013.

[30] I. Lee, E. Bender, and B. Kadenbach, "Control of mitochondrial membrane potential and ROS formation by reversible phosphorylation of cytochrome c oxidase," *Molecular and Cellular Biochemistry*, vol. 234-235, no. 1, pp. 63–70, 2002.

[31] H. Bo, W. Kang, N. Jiang, X. Wang, Y. Zhang, and L. L. Ji, "Exercise-induced neuroprotection of hippocampus in APP/PS1 transgenic mice via upregulation of mitochondrial 8-oxoguanine DNA glycosylase," *Oxidative Medicine and Cellular Longevity*, vol. 2014, Article ID 834502, 14 pages, 2014.

[32] A. M. Choi, S. W. Ryter, and B. Levine, "Autophagy in human health and disease," *The New England Journal of Medicine*, vol. 368, no. 19, pp. 1845–1846, 2013.

[33] A. M. Tolkovsky, "Mitophagy," *Biochimica et Biophysica Acta—Molecular Cell Research*, vol. 1793, no. 9, pp. 1508–1515, 2009.

[34] D. Dutta, R. Calvani, R. Bernabei, C. Leeuwenburgh, and E. Marzetti, "Contribution of impaired mitochondrial autophagy to cardiac aging: mechanisms and therapeutic opportunities," *Circulation Research*, vol. 110, no. 8, pp. 1125–1138, 2012.

[35] M. Dianat, N. Sadeghi, M. Badavi, M. Panahi, and M. T. Moghadam, "Protective effects of co-administration of gallic Acid and cyclosporine on rat myocardial morphology against ischemia/reperfusion," *Jundishapur Journal of Natural Pharmaceutical Products*, vol. 9, no. 4, Article ID e17186, 2014.

[36] S. Kobayashi and Q. Liang, "Autophagy and mitophagy in diabetic cardiomyopathy," *Biochimica et Biophysica Acta*, vol. 1852, no. 2, pp. 252–261, 2015.

[37] C. He, H. Zhu, H. Li, M.-H. Zou, and Z. Xie, "Dissociation of Bcl-2-Beclin1 complex by activated AMPK enhances cardiac autophagy and protects against cardiomyocyte apoptosis in diabetes," *Diabetes*, vol. 62, no. 4, pp. 1270–1281, 2013.

[38] S. Park, S.-G. Choi, S.-M. Yoo, J. H. Son, and Y.-K. Jung, "Choline dehydrogenase interacts with SQSTM1/p62 to recruit LC3 and stimulate mitophagy," *Autophagy*, vol. 10, no. 11, pp. 1906–1920, 2014.

[39] A. Hamacher-Brady, N. R. Brady, S. E. Logue et al., "Response to myocardial ischemia/reperfusion injury involves Bnip3 and autophagy," *Cell Death and Differentiation*, vol. 14, no. 1, pp. 146–157, 2007.

[40] R. A. Hanna, M. N. Quinsay, A. M. Orogo, K. Giang, S. Rikka, and Å. B. Gustafsson, "Microtubule-associated protein 1 light chain 3 (LC3) interacts with Bnip3 protein to selectively remove endoplasmic reticulum and mitochondria via autophagy," *The Journal of Biological Chemistry*, vol. 287, no. 23, pp. 19094–19104, 2012.

[41] R. Scherz-Shouval and Z. Elazar, "Regulation of autophagy by ROS: physiology and pathology," *Trends in Biochemical Sciences*, vol. 36, no. 1, pp. 30–38, 2011.

[42] A. G. Moyzis, J. Sadoshima, and Å. B. Gustafsson, "Mending a broken heart: the role of mitophagy in cardioprotection," *The American Journal of Physiology—Heart and Circulatory Physiology*, vol. 308, no. 3, pp. H183–H192, 2015.

[43] P. Grumati, L. Coletto, A. Schiavinato et al., "Physical exercise stimulates autophagy in normal skeletal muscles but is detrimental for collagen VI-deficient muscles," *Autophagy*, vol. 7, no. 12, pp. 1415–1423, 2011.

[44] U. Nair and D. J. Klionsky, "Activation of autophagy is required for muscle homeostasis during physical exercise," *Autophagy*, vol. 7, no. 12, pp. 1405–1406, 2011.

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Review Article

Vascular Ageing and Exercise: Focus on Cellular Reparative Processes

Mark D. Ross, Eva Malone, and Geraint Florida-James

School of Life, Sport and Social Sciences, Edinburgh Napier University, Edinburgh EH11 4BN, UK

Correspondence should be addressed to Mark D. Ross; m.ross@napier.ac.uk

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Ageing is associated with an increased risk of developing noncommunicable diseases (NCDs), such as diabetes and cardiovascular disease (CVD). The increased risk can be attributable to increased prolonged exposure to oxidative stress. Often, CVD is preceded by endothelial dysfunction, which carries with it a proatherothrombotic phenotype. Endothelial senescence and reduced production and release of nitric oxide (NO) are associated with "vascular ageing" and are often accompanied by a reduced ability for the body to repair vascular damage, termed "reendothelialization." Exercise has been repeatedly shown to confer protection against CVD and diabetes risk and incidence. Regular exercise promotes endothelial function and can prevent endothelial senescence, often through a reduction in oxidative stress. Recently, endothelial precursors, endothelial progenitor cells (EPC), have been shown to repair damaged endothelium, and reduced circulating number and/or function of these cells is associated with ageing. Exercise can modulate both number and function of these cells to promote endothelial homeostasis. In this review we look at the effects of advancing age on the endothelium and these endothelial precursors and how exercise appears to offset this "vascular ageing" process.

1. The Endothelium in Health and Disease

The endothelium controls diffusion and transport of nutrients, gases, and other signalling molecules from the blood into the surrounding tissues and controls adhesion, rolling, and migration of leukocytes to sites of infection and tissue damage. The endothelium also controls blood flow distribution around the body through the release of vasoactive substances, including nitric oxide (NO) and prostacyclin (PGI_2) [1]. Under normal conditions, NO is released from the endothelium, which diffuses to the vascular smooth muscle, causing the smooth muscle cells to relax, thus widening the diameter of the blood vessel, allowing more blood to flow distally to that vessel, a process termed endothelial function. NO is not only vasoactive to control vessel lumen diameter, but also antiatherogenic, inhibiting platelet and leukocyte adhesion to the endothelium [2].

Endothelial dysfunction often precedes CVD, and the ability of the endothelium to produce and release NO, measured as flow-mediated dilatation (FMD), can be predictive of future cardiovascular events [3] and mortality [4], potentially due to those with endothelial dysfunction being susceptible to atheroma formation and progression. Therefore the endothelium is a key player in maintenance of vascular health.

2. The Ageing Endothelium Role of Oxidative Stress

Advancing age is associated with endothelial dysfunction [5–12], increased susceptibility of endothelial cells to apoptosis [13, 14], and altered intracellular signalling [8]. These have been linked to NO bioavailability [15–17] and chronic exposure to oxidative stress [9, 18–20], which is an imbalance between the production of free radicals (oxidants) and opposing antioxidants, of which the greater production or presence of oxidants than antioxidants results in tissue damage and cellular dysfunction. Indeed the bioavailability of NO itself is a product of the rate of NO production and its scavenging by free radicals. Free radicals, such as superoxide anions ($O_2^{\bullet -}$),

have been found in greater levels in aged vascular tissue of rats compared to their younger counterparts [21], as well as in other models of ageing [22, 23], and levels of reactive oxygen species (ROS) derived from NADPH oxidase account for attenuated endothelial-dependent vasodilation in aged mice [24]. Scavenging or inhibition of these radicals improves endothelium function [15, 21]. The increased production of $O_2^{\bullet-}$ and NO leads to the formation of peroxynitrite [15, 25] and the subsequent uncoupling of eNOS [15]. In contrast to this, Luttrell et al. [26] observed high eNOS content within aortic rings of old versus young rats despite reduced endothelial function, a potential compensatory mechanism to increase the drive for NO production.

Oxidative stress can also promote atherosclerosis through the oxidation of low-density lipoprotein (oxLDL), which can stimulate macrophages to migrate from the circulation into the vascular wall [27], progressing to the development of foam cells, which is a key process in the formation of an atherosclerotic lesion. OxLDL also exerts deleterious effects on the vascular smooth muscle cell wall, by stimulating inflammatory cytokine release (tumour-necrosis factor-α, TNF-α, and monocyte chemoattractant protein-1, MCP-1) [28]. In fact, circulating oxLDL has been shown to be a predictor of cardiovascular events in humans [29], confirming its association with vascular health.

In addition to increased production of oxidants, there may be a concomitant reduction or impairment in antioxidant defences with ageing resulting in prooxidants going unchecked causing damage to surrounding tissues and accelerating ageing. For example, plasma concentration of one such antioxidant enzyme, superoxide dismutase (SOD), which itself catalyzes the dismutation of $O_2^{\bullet-}$ into oxygen and hydrogen peroxide, declines with age. This reduction was however not found in cellular tissue [11, 30], which itself may suggest that ageing may be associated with increased production of ROS rather than an impairment of cellular antioxidant capacity. Yet one study has found impaired activities of various antioxidant enzymes in aged cardiovascular tissue in rats [31]. More studies are required to fully elucidate the effects of age in animals and humans on antioxidant capacity. Acute administration of antioxidants, provided by vitamin C and SOD mimetics, can improve NO bioavailability and endothelial function [9, 32, 33], therefore offering the potential to reduce tissue damage via dietary means. This is not within the scope of this review, but for a review of this see Brown and Hu [34].

Sirtuin 1 (SIRT1) is a protein involved in DNA repair, cell cycle regulation, and ageing [35]. It functions to catalyze the removal of acetyl groups attached to lysine residues of various molecules involved in cellular signalling [36]. It is expressed in endothelial cells and has been observed to play a key role in prevention of endothelial cell senescence [37–39] by modulating p53 expression [39] and P66Shc [37], both of which are involved in oxidative stress-induced senescence. SIRT1 expression and activity are reduced in aged endothelial cells [40], but by increasing the expressing of SIRT1, endothelial senescence can be prevented [38, 41]. It appears that SIRT1 also plays a protective role in preventing ROS production in endothelial cells, as activating SIRT1 pharmacologically prevents ROS-induced endothelial dysfunction [42]. In addition, inhibition of SIRT1 caused an increase in NADPH oxidase activity and the associated $O_2^{\bullet-}$ production [42], which can go on to inactivate NO [21]. SIRT1 is therefore an important regulator of vascular endothelial ageing.

In addition to loss of NO bioavailability with ageing, there has been documented evidence of loss of prostacyclin-mediated dilatation of vasculature in humans [43] potentially as a result of reduced prostacyclin production [44, 45]. However, under NO inhibition, the dilatory response to prostacyclin in young and old subjects was similar [43], implicating loss of the NO pathway as the primary mechanism behind ageing-induced vascular dysfunction.

3. Exercise and the Endothelium

Physical activity and regular exercise training has been shown to play a significant role in the prevention of CVD, in addition to reducing the risk of mortality [46–58]. The reverse can be seen with physical inactivity and sedentary lifestyle associated with increased risk of NCD and mortality [59–68]. Physical activity and regular exercise training is antiatherogenic [69] and reduces oxidative stress through upregulation of antioxidants, such as SOD [70, 71]. Exercise has been shown to increase mitochondrial manganese SOD and cytosolic Cu/Zn SOD isoforms [72] which may contribute to a reduction in oxidative stress in the endothelium. There is a plethora of studies showing the beneficial effects of exercise on FMD, indicating improved endothelial function [5, 6, 12, 73–81]. On the other hand, sedentary interventions (reducing step counts and increasing sitting time or bed rest studies) result in the opposite effect on FMD [82–84]. Exercise and periodic increases in physical activity result in increases in cardiac output and greater blood flow through the vasculature. The increase in flow across the endothelium generates a shear stress stimulus, which is the shearing effect of circulating cells across the endothelium. Greater levels of laminar shear stress, as observed during exercise, result in an increase in NO production and release by the endothelium to widen the vessel diameter. Birk et al. [85] investigated the role of shear stress on the vascular adaptation to exercise. They observed that, in individuals who exercised for 8 weeks, brachial artery dilatory response was greater in the arm that was unrestricted to blood flow, whereas, in the arm that was restricted to blood flow via an arm inflatable cuff, there was no significant change in endothelial-dependent dilatation. Therefore shear stress is a key stimulus for vascular adaptation during an exercise training programme. The improved endothelial function can be attributable to increased endothelial NO synthase (eNOS) protein levels within the endothelium as evidenced from mice models [19, 72] and/or reduced oxidative stress [19, 72, 76, 86]. The reduced oxidative stress can help prevent the uncoupling of NO, therefore increasing NO bioavailability. One study has however shown no changes in eNOS protein content as a result of exercise training [86]. The effects of ageing and exercise on the endothelium are summarised in Figure 1.

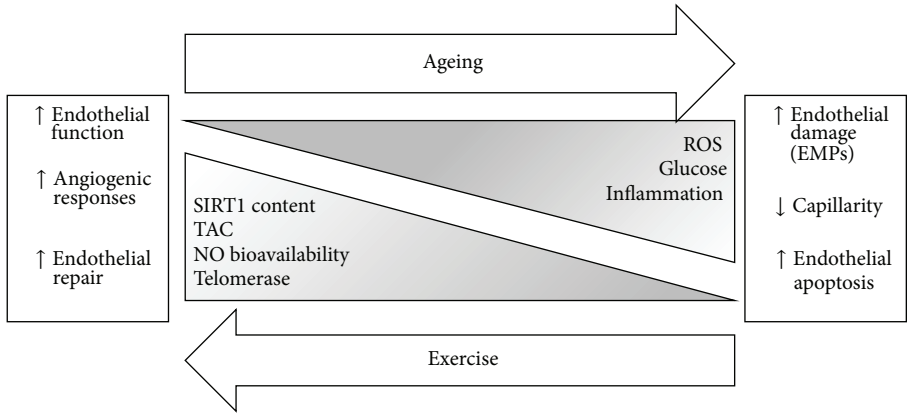

Figure 1: The effect of age and exercise on the endothelium. SIRT1: Sirtuin 1, TAC: total antioxidant capacity, NO: nitric oxide, ROS: reactive oxygen species, and EMP: endothelial microparticles.

4. Endothelial Precursors: New Cellular Markers of Endothelial Regeneration

The endothelium is reported to have a turnover rate of between 47 and 23,000 days using continuous labelling techniques [87]. However, endothelial cell turnover may be higher in areas of bifurcations [88], potentially as a result of disturbed flow [89], and increases in oxidative stress [90]. It was thought that normal endothelial cell turnover was maintained by proliferation of resident endothelial cells; however, recently, the contributions of stem cell-like cells have been described, as also seen with skin [91] and skeletal muscle [92]. Endothelial precursors or endothelial progenitor cells (EPCs) were discovered in 1997 by Asahara et al. [93]. Researchers observed that isolated $CD34^+$ cells from human peripheral blood formed tube-like structures on fibronectin-coated plates *in vitro*. These cells, after a period of 7 days in culture, began to express endothelial lineage markers such as VEGFR2, PECAM-1, and E-selectin and stained positively for eNOS. These $CD34^+$ cells also secreted NO under stimulation by vascular endothelial growth factor (VEGF) or acetylcholine, a key characteristic of mature endothelial cells. These EPCs have been consistently shown to repair damaged endothelium in animal [93–95] and human studies [96–98]; however, these endothelial precursors are rare events in human peripheral blood, accounting for only between 0.0001 and 0.01% of all mononuclear cells [99], with this level varying depending on age and health status [100]. Tissue damage may stimulate a mobilisation of $CD34^+$ progenitors, which may increase the circulating number of these cells by up to 500% (0.01% to 0.50% of all mononuclear cells) [101]. EPCs may make up a substantially smaller number in circulating pool and may only make up 10% of $CD34^+$ progenitors [102].

There is some debate on the origin of these endothelial precursors. There is clear evidence showing that they are likely derived from the bone marrow [94, 95]. Some researchers have suggested that EPCs are resident in the vessel wall, with adventitia-resident $CD34^+$ progenitors able to promote vessel formation *in vitro* [111] and *in vivo* [96]. However, Passman et al. [112] failed to observe endothelial differentiation of adventitial progenitors, with these cells instead taking a more vascular smooth muscle cell phenotype. It may be that the vascular growth and reparative process involves both circulating cells derived from the bone marrow and vascular-resident cells, promoting the proliferation of endothelial cells through paracrine means via the secretion of VEGF and also through differentiation into mature endothelial cell phenotype.

There appears to be 2 subsets of EPCs, each subset playing a different role in vascular regeneration and repair. These have been termed "early" and "late" outgrowth cells and named so because of their appearance in culture. The so-called "early" EPCs appear early in culture and die after 4 weeks. These cells secrete relatively large amounts of proangiogenic cytokines and growth factors such as VEGF and interleukin-8 (IL-8), whereas the "late" EPCs appear late in culture, live up to 12 weeks, produce more NO than "early" EPCs, and formed capillary structures to a greater extent than "early" EPCs [113]. It can be concluded that "late" EPCs have greater ability to differentiate into endothelial cells, whereas "early" EPCs have greater potential to promote vascular repair in a paracrine manner.

5. Endothelial Progenitor Cells and Vascular Disorders

Many studies have found that those with vascular-related disorders have reduced circulating number and/or impaired function of EPCs compared to healthy age-matched controls [114–138]. Numbers are also associated with endothelial function [116, 131], implicating the role of EPCs in maintaining endothelial health. Circulating "late" EPCs have also been shown to be predictive of mortality incidence, with those with higher numbers having a lower mortality rate than those with low circulating levels [139].

Paradoxically, Pelliccia et al. [140] found that those with high levels of $CD34^+CD45^-VEGFR2^+$ cells were more likely to suffer a cardiovascular event within 5 years of follow-up after undergoing percutaneous coronary intervention.

These findings may be attributable to the potential role of EPCs in the progression of atherosclerosis [126]. EPCs may contribute to atherosclerotic development through secretion of proinflammatory factors such as plasminogen activator inhibitor 1 (PAI-1) and monocyte chemoattractant protein-1 (MCP-1) [141]. Both are involved in atherosclerosis, with PAI-1 expressed within plaques, with more expression in increasingly progressed plaques [142], and with MCP-1 involved in the adhesion of monocytes to the vascular wall [143]. EPCs' secreting these proinflammatory mediators of atherosclerosis is a surprising function and paves the way for the potential role of EPCs in atherosclerosis development and progression. However there are some reports linking EPCs to the prevention of atherosclerosis, either by inverse relationships between number/function and atherosclerotic lesion development [119] or by infusion of these cells potentially causing reduced plaque burden or attenuation of plaque progression [144, 145]. Additional research is needed to fully elucidate the role in atherosclerosis development or prevention.

In the studies that show reduced circulating number of EPCs with vascular-related disorders, this reduction could be as a direct result of bone marrow depletion of these cells, due to an increased requirement for vascular repair. Mobilization of these cells by VEGF in mice has been found to reduce the number of both haematopoietic and mesenchymal stem/progenitor cells within the bone marrow after only 5 days [146]. In addition critical limb ischemia patients display reduced circulating EPCs as well as a reduced number of bone marrow resident $CD34^+$ cells compared to healthy controls [132].

The observed reduction in circulating progenitor number in those with vascular disease may also be attributable to an impaired mobilization process. Matrix metalloproteinase-9 (MMP-9) activity in the bone marrow of critical ischemia patients is reduced accompanying a reduced circulating and bone marrow resident $CD34^+$ cell number [132]. MMP-9 is involved in the mobilization of progenitors from the bone marrow [147–149], believed to be specifically involved in cleaving stromal-derived factor-1 (SDF-1, ligand for C-X-C Chemokine Receptor 4; CXCR4) allowing $CXCR4^+$ progenitor cells free to leave the bone marrow [150]. Diabetics, both type I and type II, also appear to have reduced circulating EPC numbers compared to healthy controls [120, 131, 151–156], in part due to impaired mobilization. Type II diabetics present with a reduction in capillarity within the bone marrow, which was associated with the duration of the diabetes, as well as fasting glucose levels [155], with a possible consequence of inadequate nutrient delivery for progenitor or stem cell production within the bone marrow. This could implicate impaired progenitor cell maintenance in addition to impaired mobilization.

6. Ageing and Endothelial Progenitor Cells

As discussed, ageing is associated with endothelial dysfunction, as well as impaired angiogenesis [157–161]. These effects could be associated with a reduction in EPC numbers or impaired function of these vasculogenic cells. Age does in fact result in reduced circulating EPCs [104, 106] and impaired function as displayed as reduced migration and proliferation [97, 98, 103, 105, 106, 109, 110] (Table 1). In two studies, migration and proliferation of EPCs were independent predictors of endothelial function in both young and old individuals [103, 104].

Xia et al. [97, 98] used *in vivo* mouse models to investigate the effect of age and the ability of human "early" EPCs to repair damaged endothelium. The authors induced carotid artery injury in mice and found that the ability of the mice to repair the endothelium was age-dependent. The mice that received the "young" EPCs (EPCs isolated from young individuals) displayed a greater ability to repair the endothelium in comparison to those that received the "old" EPCs (EPCs isolated from old individuals). Based on the morphological appearance of these cells being "early" EPCs, it is likely that these cells promoted reendothelialization mainly via paracrine means [113]. This *in vivo* model was accompanied by *in vitro* age-related impairments in EPC migration and adhesion of these cells. The authors reported that, under stimulation by SDF-1, these "old" cells failed to phosphorylate Janus Kinase-2 (JAK-2) to the same extent as "young" EPCs, despite similar CXCR4 cell surface expression between the two age groups, implicating a disrupted intracellular signalling mechanism as the reason by which these cells become dysfunctional, rather than cell surface protein expression changes.

EPCs from old individuals may also display impaired paracrine action, as found by Kushner et al. [108], who observed reduced release of granulocyte colony-stimulating factor (G-CSF) after stimulation by the stimulant phytohemagglutinin (PHA). However, the stimulated release of IL-8, another proangiogenic cytokine, was not different between young and old individuals. Therefore, paracrine action of these cells and their ability to stimulate endothelial repair by signalling endothelial cells to proliferate may be hindered with age.

The same group measured telomere length in EPCs isolated from peripheral blood mononuclear cells (PBMNC) in another study. Telomeres are repetitive DNA sequences (TTAGGG) at the end of chromosomes, and they act to protect DNA from damage. Replication of cells causes the length of telomeres to shorten, and therefore telomere length has often been used as a biomarker for cellular/biological age [107, 162, 163]. Repeated rounds of division and replication may cause cells to become senescent (cells are unable to replicate further). Telomere length, as measured using genomic DNA preparation and Southern hybridization techniques in EPCs isolated from PBMNC from old compared to young individuals, was shorter; however, they were not different between young and middle-aged individuals [107]. The participants in this study were reported to be healthy men, with no history of CVD or diabetes, further strengthening the belief that these cells are affected not only by disease, but also by ageing. Recent evidence suggests that telomeres can be deleteriously impacted upon by ROS. In an ageing model using *nfkb1* knockout mice, fibroblasts that show accelerated ageing also display reduction in telomere length, yet this effect was attenuated by antioxidant treatment of the mice

Table 1: Effect of age on EPC number and function.

Reference	Subject population	EPC assay	Finding
Heiss et al., 2005 [103]	20 young (~25 yr) men 20 old (~61 yr) men	$CD34^+VEGFR2^+$, $CD133^+VEGFR2^+$ cells (FC) EPC CFU EPC migration	(i) No difference in EPC number between age groups (ii) Reduced EPC migration and proliferation in old versus young men
Thijssen et al., 2006 [104]	16 young (19–28 yr) men 8 old (67–76 yr) men	$CD34^+VEGFR2^+$ cells (FC)	EPC reduced in old versus young men
Hoetzer et al., 2007 [105]	10 young (22–35 yr) men 15 middle-aged (36–55 yr) men 21 old (56–74 yr) men	EPC CFU EPC migration	(i) Reduced proliferation in middle-aged and older versus young men (ii) Reduced migration in old versus middle-aged and young men
Thum et al., 2007 [106]	10 young (23–31 yr) men 16 middle-aged (50–69 yr) men 12 old (~74 yr) men.	$CD133^+VEGFR2^+$ cells (FC) EPC migration eNOS content of EPC	EPC numbers, migration, and eNOS content reduced in old versus young and middle-aged versus young men
Kushner et al., 2009 [107]	12 young (21–34 yr), 12 middle-aged (43–55 yr), and 16 old (57–68 yr) men	Telomere length of isolated EPCs	EPC telomere length significantly reduced in older versus middle-aged and young men
Kushner et al., 2010 [108]	17 young (21–34 yr) men 20 old (56–70 yr) men	EPC release of proangiogenic factors: G-CSF, VEGF, IL-8, and IL-17	EPC release of G-CSF impaired in old versus young men
Xia et al., 2012 [97, 98]	10 young (~27 yr) men 10 old (~68 yr) men	$CD34^+VEGFR2^+$ cells (FC) Mouse model of carotid injury and infusion of EPCs from young or old men.	(i) EPC numbers reduced in old versus young men (ii) Reduced endothelial repair capacity in mouse model in old versus young men (iii) Reduced CXCR4:JAK-2 signalling in old versus young men
	25 young (~26 yr) men 22 old (~68 yr) men	As above + EPC migration EPC adhesion assay	(iv) EPC adhesion to endothelial monolayer impaired in old versus young men (v) Reduced EPC migration in old versus young men
Williamson et al., 2013 [109]	4 young (20–30 yr) individuals 4 old (50–70 yr) individuals	EPC CFU EPC migration	(i) No difference in proliferation between young and old individuals (ii) Reduced migration in old versus young individuals
Yang et al., 2013 [110]	20 young (21–33 yr) men 20 old (59–72 yr) men	$CD34^+VEGFR2^+$ cells (FC) EPC migration	Reduced number and migration of EPCs in sedentary old versus sedentary and endurance trained young, no difference in endurance trained old versus young men

FC: flow cytometry, CFU: colony forming units.

[164]. However, data is lacking with respect to circulating progenitor cells.

Once again, oxidative stress may play a central role in the ageing effect on progenitor cell number and function. $CD34^+$ progenitors in male and female octogenarians were inversely correlated with circulating levels of ROS, and those individuals who had died by the end of the follow-up (7 years) had significantly higher levels of ROS at baseline [165], highlighting the importance of reducing oxidative stress and related damage for longevity. Indeed circulating levels of ROS are greater in aged humans than young humans, and this is also accompanied by a decreased EPC content of SIRT1, which may allow ROS damage to continue unchecked. SIRT1 administration to EPCs *in vitro* rescues EPCs from H_2O_2-induced apoptosis [166], and SIRT1 deletion leads progenitor cells to exhibit an ageing phenotype as indicated by an increase in DNA damage and increased intracellular content of ROS [167]. These observations lead us to believe that the process of ageing, through the increased production of ROS, and reduced SIRT1 content of EPCs, could lead, partially, to the reduced number and function of these vascular regenerative cells, increasing risk for CVD in ageing individuals.

The evidence points to a deleterious effect of ageing on the ability of the body to stimulate endothelial repair, through depletion of EPC number, in both circulation and bone marrow, as well as impairment of function of these cells. Ageing is associated with increased risk of NCDs [168],

and the effect of age on EPCs may be a causative factor. It is therefore of great importance to maintain EPC number and function throughout the lifespan in order to reduce risk of these NCDs. There is plenty of evidence to show that pharmaceutical interventions, such as statins [169–176], can help maintain EPC number and function; however this places a large financial burden on health services, thus addressing other lifestyle factors, such as diet, and exercise may be more cost effective.

7. Exercise and Endothelial Progenitor Cells

Regular exercise has been consistently shown to be beneficial for health. Exercise can improve cardiorespiratory fitness, lower blood pressure [177, 178], improve left ventricular function [133, 179], reduce chronic low-grade inflammation [180], improve tissue perfusion [181], reduce fasting blood glucose [182], and increase insulin sensitivity [183]. Taken together, there is overwhelming evidence that regular exercise or having a higher level of cardiorespiratory fitness can offer some protection against NCD incidence and mortality [47–58]. Recently, there has been a growth in interest in EPC biology and the impact of exercise on these cells.

Acute exercise has been repeatedly shown to mobilize EPCs into the circulation in addition to enhancing the *in vitro* and *in vivo* function of these cells for a period of up to 72 hours, depending on the intensity and duration of the bout of exercise investigated [134, 184–202], with few studies showing no changes [104, 203] or even reductions in progenitors after exercise [204]. The observed increases in circulating angiogenic progenitors are often seen alongside increases in circulating SDF-1 [187], VEGF [184, 191, 193, 194], G-CSF, MMP-9 [193], or increased NO production [202]. Acute maximal exercise bout has been shown to improve the function of EPCs, as measured by increased migratory capacity to VEGF and SDF-1 *in vitro* [134] which is proposed to aid in the cells being able to migrate to ischaemic tissue to stimulate vessel growth. The improved function of these cells may be due to increases in CXCR4 cell surface expression, yet this has yet to be investigated in EPCs. Exercise-induced increases in circulating cortisol have been found to increase CXCR4 expression in T-lymphocytes [205] indicating that the circulating environment that these cells are exposed to as a result of exercise may affect cell surface receptor expression and subsequently function. Increased CXCR4 cell surface expression could also be stimulated by increases in shear stress [97] caused by increases in cardiac output seen with exercise. Further study is required to investigate the effect of acute exercise and the role CXCR4 plays in postexercise improvements in EPC migratory function. Age also appears to have an impact on the acute exercise response. EPC numbers increased in circulation in old individuals; however, this response was attenuated in comparison to a young population [200] suggesting an impaired mobilisation process.

Regular exercise training also results in increases in resting EPC numbers [98, 105, 110, 206–220], potentially contributing to the observed improvement in endothelial function with exercise [219]. However, some studies have found no changes in circulating number but did find improvements in *in vivo* endothelial-repair ability [98], *in vitro* endothelial colony forming unit ability [215], or *in vitro* NO production [218]. Xia et al. [98] investigated the effect of regular exercise training on EPC-mediated endothelial repair using a murine model of carotid artery injury. Before and after exercise training (30 minutes per day, 3 days per week, and 12 weeks of aerobic exercise) human "early" EPCs were isolated and cultured. These cells were then injected into left carotid artery of athymic nude mice after carotid injury. Endothelial regeneration was greater in the mice injected with EPCs from young subjects compared to those injected with EPCs from older subjects. Endothelial regenerative ability of these cells was improved in the older men after the 12-week training period. The improvement in EPC *in vivo* function as a result of the exercise training period in humans was associated with improvements in intracellular signalling, with increased signalling between CXCR4 and its downstream target, Janus Kinase-2 (JAK-2) [98], a potential mechanism for the improved migratory capacity of these cells after training interventions [134]. Other functional improvements seen with exercise training include improved migration to VEGF [105, 217, 220] and SDF-1 [220], adhesion to human umbilical cord vein endothelial cells [98], and secretion of NO [218]. Importantly these improvements in EPC function and/or number have been found to be related to the improvement seen in endothelial function as a result of an exercise training program [219], potentially implicating these cells in the process of improving endothelial function with exercise. The effects of age and exercise on these progenitor cell subsets and their effect on the endothelium is summarised in Figure 2.

Other mechanisms behind improved number and function of these cells with exercise training are potentially linked to reduced oxidative stress which affect progenitor cell function [166] and lower fasting blood glucose, as hyperglycaemic conditions typically affect progenitor cell functions [152, 221].

Detraining and inactivity on the other hand play a role in reducing vascular regenerative capacity of these cells. Only 10-day detraining was sufficient to reduce $CD34^+$ and $CD34^+VEGFR2^+$ progenitor cells, and the extent of decline in EPCs ($CD34^+VEGFR2^+$) was associated with the decline in endothelial function [222]. Additionally, these cells at baseline were associated with oxLDL plasma concentrations. The observed increase in EPC senescence potentially resulted from a reduction in total antioxidant capacity of the individual, which concomitantly decreased after the 10-day detraining. Data from this study suggest that oxidative stress and antioxidant capacity of the individual may be associated with physical activity and as a result may modulate EPC number and senescence and subsequent endothelial function and cardiovascular risk.

8. Summary

The process of ageing is often associated with increased morbidity and mortality. "Vascular ageing" represents

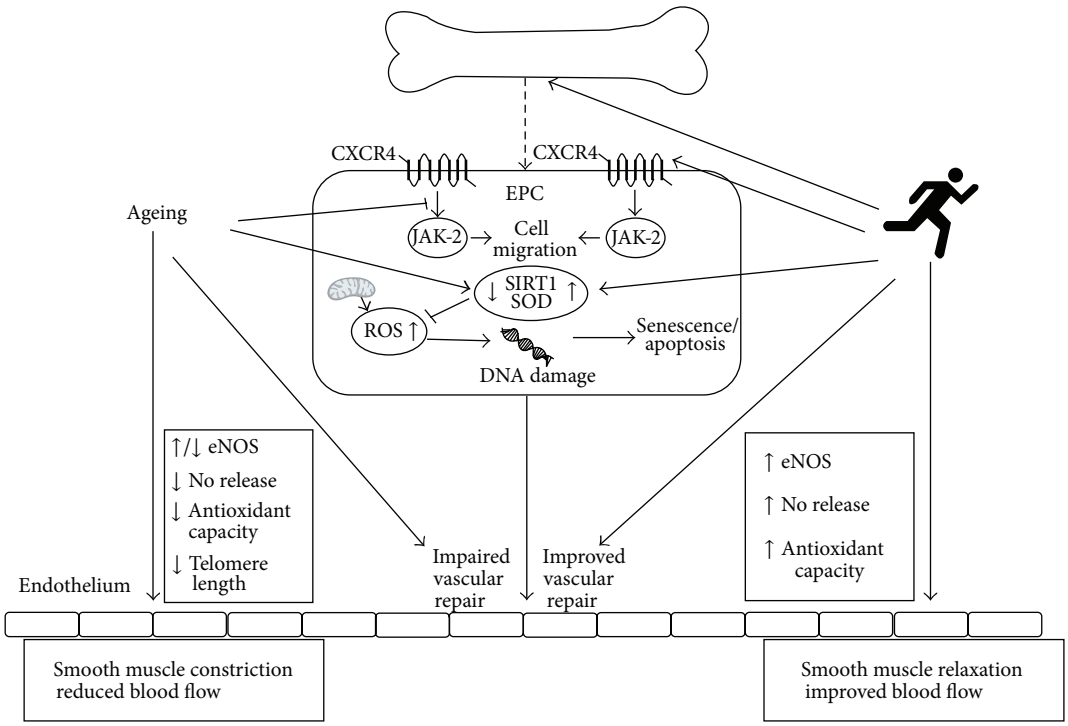

Figure 2: The effects of age and exercise on EPC-mediated vascular repair and endothelial function. Ageing causes the reduced signaling between CXCR4 and Janus Kinase-2 (JAK-2), as well as being associated with a reduced antioxidant capacity. Exercise mobilizes EPCs from bone marrow and rescues the signaling between CXCR4 and JAK-2, as well as stimulating production of antioxidants Sirtuin 1 (SIRT1) and superoxide dismutase (SOD). EPC-mediated repair of endothelium leads to improved endothelial function.

the multitude of effects of ageing on the vascular tree including endothelial dysfunction, increased arterial stiffness, atherosclerotic plaque formation, and an impaired angiogenic response. Exercise training may offset this process of "vascular ageing" by maintaining or improving EPC number and function, which can then act to help maintain endothelial function through paracrine signalling to promote endothelial proliferation or by adhering to the vessel wall and differentiating into mature endothelial cells, with fully functional eNOS and high NO content. The reduction in oxidative stress as seen following exercise training programs may also promote EPC survival and prevent functional decline of these cells with age.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] R. F. Furchgott and J. V. Zawadzki, "The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine," *Nature*, vol. 288, no. 5789, pp. 373–376, 1980.

[2] J. P. Cooke and P. S. Tsao, "Is NO an endogenous antiatherogenic molecule?" *Arteriosclerosis and Thrombosis*, vol. 14, no. 5, pp. 653–655, 1994.

[3] D. J. Green, H. Jones, D. Thijssen, N. T. Cable, and G. Atkinson, "Flow-mediated dilation and cardiovascular event prediction," *Hypertension*, vol. 57, no. 3, pp. 363–369, 2011.

[4] M. Shechter, S. Matetzky, M. Arad, M. S. Feinberg, and D. Freimark, "Vascular endothelial function predicts mortality risk in patients with advanced ischaemic chronic heart failure," *European Journal of Heart Failure*, vol. 11, no. 6, pp. 588–593, 2009.

[5] M. A. Black, N. T. Cable, D. H. J. Thijssen, and D. J. Green, "Impact of age, sex, and exercise on brachial artery flow-mediated dilatation," *The American Journal of Physiology—Heart and Circulatory Physiology*, vol. 297, no. 3, pp. H1109–H1116, 2009.

[6] M. A. Black, D. J. Green, and N. T. Cable, "Exercise prevents age-related decline in nitric-oxide-mediated vasodilator function in cutaneous microvessels," *The Journal of Physiology*, vol. 586, no. 14, pp. 3511–3524, 2008.

[7] J. M. Muller-Delp, "Aging-induced adaptations of microvascular reactivity," *Microcirculation*, vol. 13, no. 4, pp. 301–314, 2006.

[8] K. G. Soucy, S. Ryoo, A. Benjo et al., "Impaired shear stress-induced nitric oxide production through decreased NOS phosphorylation contributes to age-related vascular stiffness," *Journal of Applied Physiology*, vol. 101, no. 6, pp. 1751–1759, 2006.

[9] S. Taddei, A. Virdis, L. Ghiadoni et al., "Age-related reduction of NO availability and oxidative stress in humans," *Hypertension*, vol. 38, no. 2, pp. 274–279, 2001.

[10] R. D. Prisby, M. W. Ramsey, B. J. Behnke et al., "Aging reduces skeletal blood flow, endothelium-dependent vasodilation, and no bioavailability in rats," *Journal of Bone and Mineral Research*, vol. 22, no. 8, pp. 1280–1288, 2007.

[11] A. J. Donato, I. Eskurza, A. E. Silver et al., "Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-κB," *Circulation Research*, vol. 100, no. 11, pp. 1659–1666, 2007.

[12] S. A. Spier, M. D. Delp, C. J. Meininger, A. J. Donato, M. W. Ramsey, and J. M. Muller-Delp, "Effects of ageing and exercise training on endothelium-dependent vasodilatation and structure of rat skeletal muscle arterioles," *The Journal of Physiology*, vol. 556, no. 3, pp. 947–958, 2004.

[13] H. Wang, A. Listrat, B. Meunier et al., "Apoptosis in capillary endothelial cells in ageing skeletal muscle," *Aging Cell*, vol. 13, no. 2, pp. 254–262, 2014.

[14] J. Hoffmann, J. Haendeler, A. Aicher et al., "Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli: important role of nitric oxide," *Circulation Research*, vol. 89, no. 8, pp. 709–715, 2001.

[15] A. Csiszar, Z. Ungvari, J. G. Edwards et al., "Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function," *Circulation Research*, vol. 90, no. 11, pp. 1159–1166, 2002.

[16] M. R. Tschudi, M. Barton, N. A. Bersinger et al., "Effect of age on kinetics of nitric oxide release in rat aorta and pulmonary artery," *The Journal of Clinical Investigation*, vol. 98, no. 4, pp. 899–905, 1996.

[17] T. Tanabe, S. Maeda, T. Miyauchi et al., "Exercise training improves ageing-induced decrease in eNOS expression of the aorta," *Acta Physiologica Scandinavica*, vol. 178, no. 1, pp. 3–10, 2003.

[18] A. J. Donato, G. D. Henson, C. R. Hart et al., "The impact of ageing on adipose structure, function and vasculature in the B6D2F1 mouse: evidence of significant multisystem dysfunction," *The Journal of Physiology*, vol. 592, no. 18, pp. 4083–4096, 2014.

[19] J. R. Durrant, D. R. Seals, M. L. Connell et al., "Voluntary wheel running restores endothelial function in conduit arteries of old mice: direct evidence for reduced oxidative stress, increased superoxide dismutase activity and down-regulation of NADPH oxidase," *The Journal of Physiology*, vol. 587, no. 13, pp. 3271–3285, 2009.

[20] I. Eskurza, Z. D. Kahn, and D. R. Seals, "Xanthine oxidase does not contribute to impaired peripheral conduit artery endothelium-dependent dilatation with ageing," *The Journal of Physiology*, vol. 571, no. 3, pp. 661–668, 2006.

[21] C. A. Hamilton, M. J. Brosnan, M. McIntyre, D. Graham, and A. F. Dominiczak, "Superoxide excess in hypertension and aging: a common cause of endothelial dysfunction," *Hypertension*, vol. 37, no. 2, pp. 529–534, 2001.

[22] S. Chrissobolis and F. M. Faraci, "The role of oxidative stress and NADPH oxidase in cerebrovascular disease," *Trends in Molecular Medicine*, vol. 14, no. 11, pp. 495–502, 2008.

[23] W. G. Mayhan, D. M. Arrick, G. M. Sharpe, and H. Sun, "Age-related alterations in reactivity of cerebral arterioles: role of oxidative stress," *Microcirculation*, vol. 15, no. 3, pp. 225–236, 2008.

[24] L. Park, J. Anrather, H. Girouard, P. Zhou, and C. Iadecola, "Nox2-derived reactive oxygen species mediate neurovascular dysregulation in the aging mouse brain," *Journal of Cerebral Blood Flow and Metabolism*, vol. 27, no. 12, pp. 1908–1918, 2007.

[25] B. van der Loo, R. Labugger, J. N. Skepper et al., "Enhanced peroxynitrite formation is associated with vascular aging," *The Journal of Experimental Medicine*, vol. 192, no. 12, pp. 1731–1744, 2000.

[26] M. J. Luttrell, J. W. Seawright, E. Wilson, and C. R. Woodman, "Effect of age and exercise training on protein:protein interactions among eNOS and its regulatory proteins in rat aortas," *European Journal of Applied Physiology*, vol. 113, no. 11, pp. 2761–2768, 2013.

[27] C. Chen and D. B. Khismatullin, "Oxidized low-density lipoprotein contributes to atherogenesis via co-activation of macrophages and mast cells," *PLoS ONE*, vol. 10, no. 3, Article ID e0123088, 2015.

[28] K. Yang, X. J. Zhang, L. J. Cao et al., "Toll-like receptor 4 mediates inflammatory cytokine secretion in smooth muscle cells induced by oxidized low-density lipoprotein," *PLoS ONE*, vol. 9, no. 4, Article ID e95935, 2014.

[29] C. Meisinger, J. Baumert, N. Khuseyinova, H. Loewel, and W. Koenig, "Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population," *Circulation*, vol. 112, no. 5, pp. 651–657, 2005.

[30] M. Barton, F. Cosentino, R. P. Brandes, P. Moreau, S. Shaw, and T. F. Lüscher, "Anatomic heterogeneity of vascular aging: role of nitric oxide and endothelin," *Hypertension*, vol. 30, no. 4, pp. 817–824, 1997.

[31] F. Gündüz, Ü. K. Şentürk, O. Kuru, B. Aktekin, and M. R. Aktekin, "The effect of one year's swimming exercise on oxidant stress and antioxidant capacity in aged rats," *Physiological Research*, vol. 53, no. 2, pp. 171–176, 2004.

[32] I. Eskurza, K. D. Monahan, J. A. Robinson, and D. R. Seals, "Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing," *The Journal of Physiology*, vol. 556, no. 1, pp. 315–324, 2004.

[33] R. Tatchum-Talom and D. S. Martin, "Tempol improves vascular function in the mesenteric vascular bed of senescent rats," *Canadian Journal of Physiology and Pharmacology*, vol. 82, no. 3, pp. 200–207, 2004.

[34] A. A. Brown and F. B. Hu, "Dietary modulation of endothelial function: implications for cardiovascular disease," *American Journal of Clinical Nutrition*, vol. 73, no. 4, pp. 673–686, 2001.

[35] Y. Wang, C. Xu, Y. Liang, and P. M. Vanhoutte, "SIRT1 in metabolic syndrome: where to target matters," *Pharmacology & Therapeutics*, vol. 136, no. 3, pp. 305–318, 2012.

[36] B. Bai, P. M. Vanhoutte, and Y. Wang, "Loss-of-SIRT1 function during vascular ageing: hyperphosphorylation mediated by cyclin-dependent kinase 5," *Trends in Cardiovascular Medicine*, vol. 24, no. 2, pp. 81–84, 2014.

[37] H.-Z. Chen, Y.-Z. Wan, and D.-P. Liu, "Cross-talk between SIRT1 and p66Shc in vascular diseases," *Trends in Cardiovascular Medicine*, vol. 23, no. 7, pp. 237–241, 2013.

[38] B. Bai, Y. Liang, C. Xu et al., "Cyclin-dependent kinase 5-mediated hyperphosphorylation of sirtuin-1 contributes to the development of endothelial senescence and atherosclerosis," *Circulation*, vol. 126, no. 6, pp. 729–740, 2012.

[39] H. Ota, M. Akishita, M. Eto, K. Iijima, M. Kaneki, and Y. Ouchi, "Sirt1 modulates premature senescence-like phenotype in human endothelial cells," *Journal of Molecular and Cellular Cardiology*, vol. 43, no. 5, pp. 571–579, 2007.

[40] A. J. Donato, K. A. Magerko, B. R. Lawson, J. R. Durrant, L. A. Lesniewski, and D. R. Seals, "SIRT-1 and vascular endothelial dysfunction with ageing in mice and humans," *The Journal of Physiology*, vol. 589, no. 18, pp. 4545–4554, 2011.

[41] Y. Zu, L. Liu, M. Y. K. Lee et al., "SIRT1 promotes proliferation and prevents senescence through targeting LKB1 in primary porcine aortic endothelial cells," *Circulation Research*, vol. 106, no. 8, pp. 1384–1393, 2010.

[42] M. J. Zarzuelo, R. López-Sepúlveda, M. Sánchez et al., "SIRT1 inhibits NADPH oxidase activation and protects endothelial function in the rat aorta: Implications for vascular aging," *Biochemical Pharmacology*, vol. 85, no. 9, pp. 1288–1296, 2013.

[43] W. T. Nicholson, B. Vaa, C. Hesse, J. H. Eisenach, and M. J. Joyner, "Aging is associated with reduced prostacyclin-mediated dilation in the human forearm," *Hypertension*, vol. 53, no. 6, pp. 973–978, 2009.

[44] M. Nakajima, M. Hashimoto, F. Wang et al., "Aging decreases the production of PGI_2 in rat aortic endothelial cells," *Experimental Gerontology*, vol. 32, no. 6, pp. 685–693, 1997.

[45] E. Gomez, C. Schwendemann, S. Roger et al., "Aging and prostacyclin responses in aorta and platelets from WKY and SHR rats," *The American Journal of Physiology—Heart and Circulatory Physiology*, vol. 295, no. 5, pp. H2198–H2211, 2008.

[46] J. N. Morris, J. A. Heady, P. A. B. Raffle, C. G. Roberts, and J. W. Parks, "Coronary heart-disease and physical activity of work," *The Lancet*, vol. 262, no. 6795, pp. 1053–1057, 1953.

[47] V. W. Barry, M. Baruth, M. W. Beets, J. L. Durstine, J. Liu, and S. N. Blair, "Fitness vs. fatness on all-cause mortality: a meta-analysis," *Progress in Cardiovascular Diseases*, vol. 56, no. 4, pp. 382–390, 2014.

[48] J. D. Berry, A. Pandey, A. Gao et al., "Physical fitness and risk for heart failure and coronary artery disease," *Circulation: Heart Failure*, vol. 6, no. 4, pp. 627–634, 2013.

[49] A. K. Chomistek, D. I. Chasman, N. R. Cook, E. B. Rimm, and I.-M. Lee, "Physical activity, genes for physical fitness, and risk of coronary heart disease," *Medicine and Science in Sports & Exercise*, vol. 45, no. 4, pp. 691–697, 2013.

[50] D. J. Duncker, E. D. van Deel, M. C. de Waard, M. de Boer, D. Merkus, and J. van der Velden, "Exercise training in adverse cardiac remodeling," *Pflugers Archiv European Journal of Physiology*, vol. 466, no. 6, pp. 1079–1091, 2014.

[51] A. Holtermann, J. L. Marott, F. Gyntelberg et al., "Self–reported cardiorespiratory fitness: prediction and classification of risk of cardiovascular disease mortality and longevity—a prospective investigation in the Copenhagen City Heart Study," *Journal of the American Heart Association*, vol. 4, no. 1, 2014.

[52] M. J. Joyner and D. J. Green, "Exercise protects the cardiovascular system: effects beyond traditional risk factors," *The Journal of Physiology*, vol. 587, no. 23, pp. 5551–5558, 2009.

[53] S. Kurl, J. A. Laukkanen, R. Rauramaa, T. A. Lakka, J. Sivenius, and J. T. Salonen, "Cardiorespiratory fitness and the risk for stroke in men," *Archives of Internal Medicine*, vol. 163, no. 14, pp. 1682–1688, 2003.

[54] I.-M. Lee, E. J. Shiroma, F. Lobelo et al., "Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy," *The Lancet*, vol. 380, no. 9838, pp. 219–229, 2012.

[55] P. Schnohr, J. H. O'Keefe, J. L. Marott, P. Lange, and G. B. Jensen, "Dose of jogging and long-term mortality: the Copenhagen City Heart Study," *Journal of the American College of Cardiology*, vol. 65, no. 5, pp. 411–419, 2015.

[56] D. L. Swift, C. J. Lavie, N. M. Johannsen et al., "Physical activity, cardiorespiratory fitness, and exercise training in primary and secondary coronary prevention," *Circulation Journal*, vol. 77, no. 2, pp. 281–292, 2013.

[57] R. Vigen, C. Ayers, B. Willis, L. DeFina, and J. D. Berry, "Association of cardiorespiratory fitness with total, cardiovascular, and noncardiovascular mortality across 3 decades of follow-up in men and women," *Circulation: Cardiovascular Quality and Outcomes*, vol. 5, no. 3, pp. 358–364, 2012.

[58] M. A. West, D. Lythgoe, C. P. Barben et al., "Cardiopulmonary exercise variables are associated with postoperative morbidity after major colonic surgery: a prospective blinded observational study," *British Journal of Anaesthesia*, vol. 112, no. 4, pp. 665–671, 2014.

[59] J. Y. Chau, A. Grunseit, K. Midthjell et al., "Sedentary behaviour and risk of mortality from all-causes and cardiometabolic diseases in adults: evidence from the HUNT3 population cohort," *British Journal of Sports Medicine*, vol. 49, pp. 737–742, 2015.

[60] B. B. Gibbs, J. P. Reis, E. B. Schelbert et al., "Sedentary screen time and left ventricular structure and function: the CARDIA study," *Medicine and Science in Sports & Exercise*, vol. 46, no. 2, pp. 276–283, 2014.

[61] M. T. Hamilton, D. G. Hamilton, and T. W. Zderic, "Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease," *Diabetes*, vol. 56, no. 11, pp. 2655–2667, 2007.

[62] P. T. Katzmarzyk, T. S. Church, C. L. Craig, and C. Bouchard, "Sitting time and mortality from all causes, cardiovascular disease, and cancer," *Medicine & Science in Sports & Exercise*, vol. 41, no. 5, pp. 998–1005, 2009.

[63] U. Laufs, S. Wassmann, T. Czech et al., "Physical inactivity increases oxidative stress, endothelial dysfunction, and atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 4, pp. 809–814, 2005.

[64] A. E. Staiano, D. M. Harrington, T. V. Barreira, and P. T. Katzmarzyk, "Sitting time and cardiometabolic risk in US adults: associations by sex, race, socioeconomic status and activity level," *British Journal of Sports Medicine*, vol. 48, no. 3, pp. 213–219, 2014.

[65] E. Stamatakis, J. Y. Chau, Z. Pedisic et al., "Are sitting occupations associated with increased all-cause, cancer, and cardiovascular disease mortality risk? A pooled analysis of seven british population cohorts," *PLoS ONE*, vol. 8, no. 9, Article ID e73753, 2013.

[66] H. P. van der Ploeg, T. Chey, R. J. Korda, E. Banks, and A. Bauman, "Sitting time and all-cause mortality risk in 222 497 Australian adults," *Archives of Internal Medicine*, vol. 172, no. 6, pp. 494–500, 2012.

[67] E. G. Wilmot, C. L. Edwardson, F. A. Achana et al., "Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis," *Diabetologia*, vol. 55, no. 11, pp. 2895–2905, 2012.

[68] D. R. Young, K. Reynolds, M. Sidell et al., "Effects of physical activity and sedentary time on the risk of heart failure," *Circulation: Heart Failure*, vol. 7, no. 1, pp. 21–27, 2014.

[69] J. Szostak and P. Laurant, "The forgotten face of regular physical exercise: a 'natural' anti-atherogenic activity," *Clinical Science*, vol. 121, no. 3, pp. 91–106, 2011.

[70] H. Miyazaki, S. Oh-ishi, T. Ookawara et al., "Strenuous endurance training in humans reduces oxidative stress following exhausting exercise," *European Journal of Applied Physiology*, vol. 84, no. 1-2, pp. 1–6, 2001.

[71] T. Fukai, M. R. Siegfried, M. Ushio-Fukai, Y. Cheng, G. Kojda, and D. G. Harrison, "Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training,"

Journal of Clinical Investigation, vol. 105, no. 11, pp. 631–1639, 2000.

[72] F. Moien-Afshari, S. Ghosh, M. Khazaei, T. J. Kieffer, R. W. Brownsey, and I. Laher, "Exercise restores endothelial function independently of weight loss or hyperglycaemic status in db/db mice," *Diabetologia*, vol. 51, no. 7, pp. 1327–1337, 2008.

[73] A. W. Ashor, J. Lara, M. Siervo et al., "Exercise modalities and endothelial function: a systematic review and dose—response meta-analysis of randomized controlled trials," *Sports Medicine*, vol. 45, no. 2, pp. 279–296, 2014.

[74] G. K. Birk, E. A. Dawson, A. M. Batterham et al., "Effects of exercise intensity on flow mediated dilation in healthy humans," *International Journal of Sports Medicine*, vol. 34, no. 5, pp. 409–414, 2013.

[75] F. Farsidfar, E. Kasikcioglu, H. Oflaz, D. Kasikcioglu, M. Meric, and S. Umman, "Effects of different intensities of acute exercise on flow-mediated dilatation in patients with coronary heart disease," *International Journal of Cardiology*, vol. 124, no. 3, pp. 372–374, 2008.

[76] C. Goto, Y. Higashi, M. Kimura et al., "Effect of different intensities of exercise on endothelium-dependent vasodilation in humans," *Circulation*, vol. 108, no. 5, pp. 530–535, 2003.

[77] T.-H. Luk, Y.-L. Dai, C.-W. Siu et al., "Effect of exercise training on vascular endothelial function in patients with stable coronary artery disease: a randomized controlled trial," *European Journal of Preventive Cardiology*, vol. 19, no. 4, pp. 830–839, 2012.

[78] W. Mitranun, C. Deerochanawong, H. Tanaka, and D. Suksom, "Continuous vs interval training on glycemic control and macro- and microvascular reactivity in type 2 diabetic patients," *Scandinavian Journal of Medicine & Science in Sports*, vol. 24, no. 2, pp. e69–e76, 2014.

[79] J. M. Murias, K. N. Grise, M. Jiang, H. Kowalchuk, C. W. J. Melling, and E. G. Noble, "Acute endurance exercise induces changes in vasorelaxation responses that are vessel-specific," *The American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 304, no. 7, pp. R574–R580, 2013.

[80] M. Rakobowchuk, S. Tanguay, K. A. Burgomaster, K. R. Howarth, M. J. Gibala, and M. J. MacDonald, "Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow-mediated dilation in healthy humans," *American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 295, no. 1, pp. R236–R242, 2008.

[81] T. M. Tinken, D. H. J. Thijssen, N. Hopkins, E. A. Dawson, N. T. Cable, and D. J. Green, "Shear stress mediates endothelial adaptations to exercise training in humans," *Hypertension*, vol. 55, no. 2, pp. 312–318, 2010.

[82] S. S. Thosar, B. D. Johnson, J. D. Johnston, and J. P. Wallace, "Sitting and endothelial dysfunction: the role of shear stress," *Medical Science Monitor*, vol. 18, no. 12, pp. 173–180, 2012.

[83] S. S. Thosar, S. L. Bielko, K. J. Mather, J. D. Johnston, and J. P. Wallace, "Effect of prolonged sitting and breaks in sitting time on endothelial function," *Medicine & Science in Sports & Exercise*, vol. 47, no. 4, pp. 843–849, 2015.

[84] C. Demiot, F. Dignat-George, J.-O. Fortrat et al., "WISE 2005: chronic bed rest impairs microcirculatory endothelium in women," *The American Journal of Physiology—Heart and Circulatory Physiology*, vol. 293, no. 5, pp. H3159–H3164, 2007.

[85] G. K. Birk, E. A. Dawson, C. Atkinson et al., "Brachial artery adaptation to lower limb exercise training: role of shear stress," *Journal of Applied Physiology*, vol. 112, no. 10, pp. 1653–1658, 2012.

[86] S. Lee, Y. Park, K. C. Dellsperger, and C. Zhang, "Exercise training improves endothelial function via adiponectin-dependent and independent pathways in type 2 diabetic mice," *The American Journal of Physiology—Heart and Circulatory Physiology*, vol. 301, no. 2, pp. H306–H314, 2011.

[87] B. Hobson and J. Denekamp, "Endothelial proliferation in tumours and normal tissues: continuous labelling studies," *British Journal of Cancer*, vol. 49, no. 4, pp. 405–413, 1984.

[88] B. A. Caplan and C. J. Schwartz, "Increased endothelial cell turnover in areas of in vivo Evans Blue uptake in the pig aorta," *Atherosclerosis*, vol. 17, no. 3, pp. 401–417, 1973.

[89] C. M. Warboys, A. de Luca, N. Amini et al., "Disturbed flow promotes endothelial senescence via a p53-dependent pathway," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 34, no. 5, pp. 985–995, 2014.

[90] J. D. Erusalimsky, "Vascular endothelial senescence: from mechanisms to pathophysiology," *Journal of Applied Physiology*, vol. 106, no. 1, pp. 326–332, 2009.

[91] C. Blanpain and E. Fuchs, "Epidermal stem cells of the skin," *Annual Review of Cell and Developmental Biology*, vol. 22, pp. 339–373, 2006.

[92] X. Shi and D. J. Garry, "Muscle stem cells in development, regeneration, and disease," *Genes & Development*, vol. 20, no. 13, pp. 1692–1708, 2006.

[93] T. Asahara, T. Murohara, A. Sullivan et al., "Isolation of putative progenitor endothelial cells for angiogenesis," *Science*, vol. 275, no. 5302, pp. 964–967, 1997.

[94] T. Asahara, H. Masuda, T. Takahashi et al., "Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization," *Circulation Research*, vol. 85, no. 3, pp. 221–228, 1999.

[95] M. Reyes, A. Dudek, B. Jahagirdar, L. Koodie, P. H. Marker, and C. M. Verfaillie, "Origin of endothelial progenitors in human postnatal bone marrow," *The Journal of Clinical Investigation*, vol. 109, no. 3, pp. 337–346, 2002.

[96] E. Zengin, F. Chalajour, U. M. Gehling et al., "Vascular wall resident progenitor cells: a source for postnatal vasculogenesis," *Development*, vol. 133, no. 8, pp. 1543–1551, 2006.

[97] W. H. Xia, Z. Yang, S. Y. Xu et al., "Age-related decline in reendothelialization capacity of human endothelial progenitor cells is restored by shear stress," *Hypertension*, vol. 59, no. 6, pp. 1225–1231, 2012.

[98] W.-H. Xia, J. Li, C. Su et al., "Physical exercise attenuates age-associated reduction in endothelium-reparative capacity of endothelial progenitor cells by increasing CXCR4/JAK-2 signaling in healthy men," *Aging Cell*, vol. 11, no. 1, pp. 111–119, 2012.

[99] J. Case, L. E. Mead, W. K. Bessler et al., "Human $CD34^+AC133^+VEGFR\text{-}2^+$ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors," *Experimental Hematology*, vol. 35, no. 7, pp. 1109–1118, 2007.

[100] K. S. Cohen, S. Cheng, M. G. Larson et al., "Circulating $CD34^+$ progenitor cell frequency is associated with clinical and genetic factors," *Blood*, vol. 121, no. 8, pp. e50–e56, 2013.

[101] U. Ritz, V. Spies, I. Mehling, D. Gruszka, P. M. Rommens, and A. Hofmann, "Mobilization of $CD34^+$-progenitor cells in patients with severe trauma," *PLoS ONE*, vol. 9, no. 5, Article ID e97369, 2014.

[102] R. S. Patel, Q. Li, N. Ghasemzadeh et al., "Circulating $CD34^+$ progenitor cells and risk of mortality in a population with coronary artery disease," *Circulation Research*, vol. 116, no. 2, pp. 289–297, 2015.

[103] C. Heiss, S. Keymel, U. Niesler, J. Ziemann, M. Kelm, and C. Kalka, "Impaired progenitor cell activity in age-related endothelial dysfunction," *Journal of the American College of Cardiology*, vol. 45, no. 9, pp. 1441–1448, 2005.

[104] D. H. J. Thijssen, J. B. Vos, C. Verseyden et al., "Haematopoietic stem cells and endothelial progenitor cells in healthy men: effect of aging and training," *Aging Cell*, vol. 5, no. 6, pp. 495–503, 2006.

[105] G. L. Hoetzer, G. P. Van Guilder, H. M. Irmiger, R. S. Keith, B. L. Stauffer, and C. A. DeSouza, "Aging, exercise, and endothelial progenitor cell clonogenic and migratory capacity in men," *Journal of Applied Physiology*, vol. 102, no. 3, pp. 847–852, 2007.

[106] T. Thum, S. Hoeber, S. Froese et al., "Age-dependent impairment of endothelial progenitor cells is corrected by growth hormone mediated increase of insulin-like growth factor-1," *Circulation Research*, vol. 100, no. 3, pp. 434–443, 2007.

[107] E. J. Kushner, G. P. Van Guilder, O. J. MacEneaney, J. N. Cech, B. L. Stauffer, and C. A. DeSouza, "Aging and endothelial progenitor cell telomere length in healthy men," *Clinical Chemistry and Laboratory Medicine*, vol. 47, no. 1, pp. 47–50, 2009.

[108] E. Kushner, G. Van Guilder, O. MacEneaney et al., "Ageing and endothelial progenitor cell release of proangiogenic cytokines," *Age and Ageing*, vol. 39, no. 2, Article ID afp244, pp. 268–272, 2010.

[109] K. A. Williamson, A. Hamilton, J. A. Reynolds et al., "Age-related impairment of endothelial progenitor cell migration correlates with structural alterations of heparan sulfate proteoglycans," *Aging Cell*, vol. 12, no. 1, pp. 139–147, 2013.

[110] Z. Yang, W.-H. Xia, C. Su et al., "Regular exercise-induced increased number and activity of circulating endothelial progenitor cells attenuates age-related decline in arterial elasticity in healthy men," *International Journal of Cardiology*, vol. 165, no. 2, pp. 247–254, 2013.

[111] G. Pasquinelli, P. L. Tazzari, C. Vaselli et al., "Thoracic aortas from multiorgan donors are suitable for obtaining resident angiogenic mesenchymal stromal cells," *Stem Cells*, vol. 25, no. 7, pp. 1627–1634, 2007.

[112] J. N. Passman, X. R. Dong, S.-P. Wu et al., "A sonic hedgehog signaling domain in the arterial adventitia supports resident $Sca1^+$ smooth muscle progenitor cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 27, pp. 9349–9354, 2008.

[113] J. Hur, C.-H. Yoon, H.-S. Kim et al., "Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 2, pp. 288–293, 2004.

[114] M. C. Barsotti, T. Santoni, M. E. L. Picoi et al., "Endothelial progenitor cell homing in human myocardium in patients with coronary artery disease," *International Journal of Cardiology*, vol. 172, no. 2, pp. 516–517, 2014.

[115] A. E. Berezin and A. A. Kremzer, "Circulating endothelial progenitor cells as markers for severity of ischemic chronic heart failure," *Journal of Cardiac Failure*, vol. 20, no. 6, pp. 438–447, 2014.

[116] L. Bruyndonckx, V. Y. Hoymans, G. Frederix et al., "Endothelial progenitor cells and endothelial microparticles are independent predictors of endothelial function," *The Journal of Pediatrics*, vol. 165, no. 2, pp. 300–305, 2014.

[117] R. Castejon, C. Jimenez-Ortiz, S. Valero-Gonzalez, S. Rosado, S. Mellor, and M. Yebra-Bango, "Decreased circulating endothelial progenitor cells as an early risk factor of subclinical atherosclerosis in systemic lupus erythematosus," *Rheumatology*, vol. 53, no. 4, pp. 631–638, 2014.

[118] K. H. Chan, P. J. L. Simpson, A. S. Yong et al., "The relationship between endothelial progenitor cell populations and epicardial and microvascular coronary disease—a cellular, angiographic and physiologic study," *PLoS ONE*, vol. 9, no. 4, Article ID e93980, 2014.

[119] G. P. Fadini, A. Coracina, I. Baesso et al., "Peripheral blood $CD34^+KDR^+$ endothelial progenitor cells are determinants of subclinical atherosclerosis in a middle-aged general population," *Stroke*, vol. 37, no. 9, pp. 2277–2282, 2006.

[120] G. P. Fadini, M. Miorin, M. Facco et al., "Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus," *Journal of the American College of Cardiology*, vol. 45, no. 9, pp. 1449–1457, 2005.

[121] J. M. Hill, G. Zalos, J. P. J. Halcox et al., "Circulating endothelial progenitor cells, vascular function, and cardiovascular risk," *The New England Journal of Medicine*, vol. 348, no. 7, pp. 593–600, 2003.

[122] C. Jung, A. Rafnsson, A. Shemyakin, F. Böhm, and J. Pernow, "Different subpopulations of endothelial progenitor cells and circulating apoptotic progenitor cells in patients with vascular disease and diabetes," *International Journal of Cardiology*, vol. 143, no. 3, pp. 368–372, 2010.

[123] S. A. Kocaman, M. R. Yalçın, M. Yağcı et al., "Endothelial progenitor cells (CD34+KDR+) and monocytes may provide the development of good coronary collaterals despite the vascular risk factors and extensive atherosclerosis," *Anadolu Kardiyoloji Dergisi*, vol. 11, no. 4, pp. 290–299, 2011.

[124] Y.-F. Liao, Y. Feng, L.-L. Chen, T.-S. Zeng, F. Yu, and L.-J. Hu, "Coronary heart disease risk equivalence in diabetes and arterial diseases characterized by endothelial function and endothelial progenitor cell," *Journal of Diabetes and its Complications*, vol. 28, no. 2, pp. 214–218, 2014.

[125] X. Liu and C. Xie, "Human endothelial progenitor cells isolated from COPD patients are dysfunctional," *Molecular and Cellular Biochemistry*, vol. 363, no. 1-2, pp. 53–63, 2012.

[126] A. Massot, M. Navarro-Sobrino, A. Penalba et al., "Decreased levels of angiogenic growth factors in intracranial atherosclerotic disease despite severity-related increase in endothelial progenitor cell counts," *Cerebrovascular Diseases*, vol. 35, no. 1, pp. 81–88, 2013.

[127] T. M. Powell, J. D. Paul, J. M. Hill et al., "Granulocyte colony-stimulating factor mobilizes functional endothelial progenitor cells in patients with coronary artery disease," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 2, pp. 296–301, 2005.

[128] R. P. W. Rouhl, A. E. C. S. Mertens, R. J. van Oostenbrugge et al., "Angiogenic T-cells and putative endothelial progenitor cells in hypertension-related cerebral small vessel disease," *Stroke*, vol. 43, no. 1, pp. 256–258, 2012.

[129] C. Schmidt-Lucke, L. Rössig, S. Fichtlscherer et al., "Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair," *Circulation*, vol. 111, no. 22, pp. 2981–2987, 2005.

[130] E. Shantsila, B. J. Wrigley, A. Shantsila, L. D. Tapp, P. S. Gill, and G. Y. H. Lip, "Monocyte-derived and $CD34^+/KDR^+$ endothelial progenitor cells in heart failure," *Journal of Thrombosis and Haemostasis*, vol. 10, no. 7, pp. 1252–1261, 2012.

[131] L. Sibal, A. Aldibbiat, S. C. Agarwal et al., "Circulating endothelial progenitor cells, endothelial function, carotid intima–media thickness and circulating markers of endothelial dysfunction in people with type 1 diabetes without macrovascular disease or microalbuminuria," *Diabetologia*, vol. 52, no. 8, pp. 1464–1473, 2009.

[132] M. Teraa, R. W. Sprengers, P. E. Westerweel et al., "Bone marrow alterations and lower endothelial progenitor cell numbers in critical limb ischemia patients," *PLoS ONE*, vol. 8, no. 1, Article ID e55592, 2013.

[133] R. G. Turan, M. Brehm, M. Köstering et al., "Effects of exercise training on mobilization of BM-CPCs and migratory capacity as well as LVEF after AMI," *Medizinische Klinik*, vol. 101, pp. 198–201, 2006.

[134] E. M. Van Craenenbroeck, P. J. Beckers, N. M. Possemiers et al., "Exercise acutely reverses dysfunction of circulating angiogenic cells in chronic heart failure," *European Heart Journal*, vol. 31, no. 15, pp. 1924–1934, 2010.

[135] M. Vasa, S. Fichtlscherer, A. Aicher et al., "Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease," *Circulation Research*, vol. 89, no. 1, pp. e1–e7, 2001.

[136] K. Vemparala, A. Roy, V. K. Bahl et al., "Early accelerated senescence of circulating endothelial progenitor cells in premature coronary artery disease patients in a developing country—a case control study," *BMC Cardiovascular Disorders*, vol. 13, article 104, 2013.

[137] D. H. Walter, J. Haendeler, J. Reinhold et al., "Impaired CXCR4 signaling contributes to the reduced neovascularization capacity of endothelial progenitor cells from patients with coronary artery disease," *Circulation Research*, vol. 97, no. 11, pp. 1142–1151, 2005.

[138] Q. Xiao, S. Kiechi, S. Patel et al., "Endothelial progenitor cells, cardiovascular risk factors, cytokine levels and atherosclerosis—results from a large population-based study," *PLoS ONE*, vol. 2, no. 10, article e975, 2007.

[139] A. C. Alba, S. D. Lalonde, V. Rao, S. D. Walter, G. H. Guyatt, and H. J. Ross, "Changes in circulating progenitor cells are associated with outcome in heart failure patients: a longitudinal study," *Canadian Journal of Cardiology*, vol. 29, no. 12, pp. 1657–1664, 2013.

[140] F. Pelliccia, V. Pasceri, G. Rosano et al., "Endothelial progenitor cells predict long-term prognosis in patients with stable angina treated with percutaneous coronary intervention: five-year follow-up of the PROCREATION Study," *Circulation Journal*, vol. 77, no. 7, pp. 1728–1735, 2013.

[141] Y. Zhang, D. A. Ingram, M. P. Murphy et al., "Release of proinflammatory mediators and expression of proinflammatory adhesion molecules by endothelial progenitor cells," *American Journal of Physiology—Heart and Circulatory Physiology*, vol. 296, no. 5, pp. H1675–H1682, 2009.

[142] T. Padró, M. Steins, C.-X. Li et al., "Comparative analysis of plasminogen activator inhibitor-1 expression in different types of atherosclerotic lesions in coronary arteries from human heart explants," *Cardiovascular Research*, vol. 36, no. 1, pp. 28–36, 1997.

[143] R. E. Gerszten, E. A. Garcia-Zepeda, Y.-C. Lim et al., "MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions," *Nature*, vol. 398, no. 6729, pp. 718–725, 1999.

[144] D. Tousoulis, A. Briasoulis, G. Vogiatzi et al., "Effects of direct infusion of bone marrow-derived progenitor cells and indirect mobilization of hematopoietic progenitor cells on atherosclerotic plaque and inflammatory process in atherosclerosis," *International Journal of Cardiology*, vol. 168, no. 5, pp. 4769–4774, 2013.

[145] L. Yao, J. Heuser-Baker, O. Herlea-Pana et al., "Bone marrow endothelial progenitors augment atherosclerotic plaque regression in a mouse model of plasma lipid lowering," *Stem Cells*, vol. 30, no. 12, pp. 2720–2731, 2012.

[146] K. Tashiro, A. Nonaka, N. Hirata, T. Yamaguchi, H. Mizuguchi, and K. Kawabata, "Plasma elevation of vascular endothelial growth factor leads to the reduction of mouse hematopoietic and mesenchymal stem/progenitor cells in the bone marrow," *Stem Cells and Development*, vol. 23, no. 18, pp. 2202–2210, 2014.

[147] P.-H. Huang, Y.-H. Chen, C.-H. Wang et al., "Matrix metalloproteinase-9 is essential for ischemia-induced neovascularization by modulating bone marrow–derived endothelial progenitor cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 8, pp. 1179–1184, 2009.

[148] K. Jujo, H. Hamada, A. Iwakura et al., "CXCR4 blockade augments bone marrow progenitor cell recruitment to the neovasculature and reduces mortality after myocardial infarction," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 24, pp. 11008–11013, 2010.

[149] L. Ling, Y. Shen, K. Wang et al., "Worse clinical outcomes in acute myocardial infarction patients with type 2 diabetes mellitus: relevance to impaired endothelial progenitor cells mobilization," *PLoS ONE*, vol. 7, no. 11, Article ID e50739, 2012.

[150] F. Jin, Q. Zhai, L. Qiu et al., "Degradation of BM SDF-1 by MMP-9: the role in G-CSF-induced hematopoietic stem/progenitor cell mobilization," *Bone Marrow Transplantation*, vol. 42, no. 9, pp. 581–588, 2008.

[151] S. Hamed, B. Brenner, A. Aharon, D. Daoud, and A. Roguin, "Nitric oxide and superoxide dismutase modulate endothelial progenitor cell function in type 2 diabetes mellitus," *Cardiovascular Diabetology*, vol. 8, no. 1, article 56, 2009.

[152] C. Jiraritthamrong, P. Kheolamai, Y. U-Pratya et al., "In vitro vessel-forming capacity of endothelial progenitor cells in high glucose conditions," *Annals of Hematology*, vol. 91, no. 3, pp. 311–320, 2012.

[153] M. F. Lombardo, P. Iacopino, M. Cuzzola et al., "Type 2 diabetes mellitus impairs the maturation of endothelial progenitor cells and increases the number of circulating endothelial cells in peripheral blood," *Cytometry Part A*, vol. 81, no. 10, pp. 856–864, 2012.

[154] S. Meng, J. Cao, X. Zhang et al., "Downregulation of microRNA-130a contributes to endothelial progenitor cell dysfunction in diabetic patients via its target Runx3," *PLoS ONE*, vol. 8, no. 7, Article ID e68611, 2013.

[155] G. Spinetti, D. Cordella, O. Fortunato et al., "Global remodeling of the vascular stem cell niche in bone marrow of diabetic patients: implication of the microRNA-155/FOXO3a signaling pathway," *Circulation Research*, vol. 112, no. 3, pp. 510–522, 2013.

[156] W.-S. Yue, K.-K. Lau, C.-W. Siu et al., "Impact of glycemic control on circulating endothelial progenitor cells and arterial stiffness in patients with type 2 diabetes mellitus," *Cardiovascular Diabetology*, vol. 10, no. 1, article 113, 2011.

[157] A. G. Gunin, V. V. Petrov, N. N. Golubtzova, O. V. Vasilieva, and N. K. Kornilova, "Age-related changes in angiogenesis in human dermis," *Experimental Gerontology*, vol. 55, no. 1, pp. 143–151, 2014.

[158] M. J. Reed and J. M. Edelberg, "Impaired angiogenesis in the aged," *Science of Aging Knowledge Environment*, vol. 2004, no. 7, article pe7, 2004.

[159] A. Rivard, J.-E. Fabre, M. Silver et al., "Age-dependent impairment of angiogenesis," *Circulation*, vol. 99, no. 1, pp. 111–120, 1999.

[160] E. Sadoun and M. J. Reed, "Impaired angiogenesis in aging is associated with alterations in vessel density, matrix composition, inflammatory response, and growth factor expression," *Journal of Histochemistry & Cytochemistry*, vol. 51, no. 9, pp. 1119–1130, 2003.

[161] J. Wang, X. Peng, R. M. Lassance-Soares et al., "Aging-induced collateral dysfunction: Impaired responsiveness of collaterals and susceptibility to apoptosis via dysfunctional eNOS signaling," *Journal of Cardiovascular Translational Research*, vol. 4, no. 6, pp. 779–789, 2011.

[162] I. B. Ø. Østhus, A. Sgura, F. Berardinelli et al., "Telomere length and long-term endurance exercise: does exercise training affect biological age? A pilot study," *PLoS ONE*, vol. 7, no. 12, Article ID e52769, 2012.

[163] E. Chang and C. B. Harley, "Telomere length and replicative aging in human vascular tissues," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 24, pp. 11190–11194, 1995.

[164] D. Jurk, C. Wilson, J. F. Passos et al., "Chronic inflammation induces telomere dysfunction and accelerates ageing in mice," *Nature Communications*, vol. 2, article 4172, 2014.

[165] G. Mandraffino, M. A. Sardo, S. Riggio et al., "Circulating progenitor cells and the elderly: a seven-year observational study," *Experimental Gerontology*, vol. 47, no. 5, pp. 394–400, 2012.

[166] Y. Wang, Q. Cao, F. Wang et al., "SIRT1 protects against oxidative stress-induced endothelial progenitor cells apoptosis by inhibiting FOXO3a via FOXO3a ubiquitination and degradation," *Journal of Cellular Physiology*, vol. 230, no. 9, pp. 2098–2107, 2015.

[167] P. Rimmelé, C. L. Bigarella, R. Liang et al., "Aging-like phenotype and defective lineage specification in SIRT1-deleted hematopoietic stem and progenitor cells," *Stem Cell Reports*, vol. 3, no. 1, pp. 44–59, 2014.

[168] W. B. Kannel and T. Gordon, "Evaluation of cardiovascular risk in the elderly: the Framingham study," *Bulletin of the New York Academy of Medicine*, vol. 54, no. 6, pp. 573–591, 1978.

[169] J. Zhou, M. Cheng, Y.-H. Liao et al., "Rosuvastatin enhances angiogenesis via eNOS-dependent mobilization of endothelial progenitor cells," *PLoS ONE*, vol. 8, no. 5, Article ID e63126, 2013.

[170] H. Ye, F. He, X. Fei et al., "High-dose atorvastatin reloading before percutaneous coronary intervention increased circulating endothelial progenitor cells and reduced inflammatory cytokine expression during the perioperative period," *Journal of Cardiovascular Pharmacology and Therapeutics*, vol. 19, no. 3, pp. 290–295, 2014.

[171] D. H. Walter, K. Rittig, F. H. Bahlmann et al., "Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells," *Circulation*, vol. 105, no. 25, pp. 3017–3024, 2002.

[172] I. Spyridopoulos, J. Haendeler, C. Urbich et al., "Statins enhance migratory capacity by upregulation of the telomere repeat-binding factor TRF2 in endothelial progenitor cells," *Circulation*, vol. 110, no. 19, pp. 3136–3142, 2004.

[173] H. Shao, Y. Tan, D. Eton et al., "Statin and stromal cell-derived factor-1 additively promote angiogenesis by enhancement of progenitor cells incorporation into new vessels," *Stem Cells*, vol. 26, no. 5, pp. 1376–1384, 2008.

[174] E. Oikonomou, G. Siasos, M. Zaromitidou et al., "Atorvastatin treatment improves endothelial function through endothelial progenitor cells mobilization in ischemic heart failure patients," *Atherosclerosis*, vol. 238, no. 2, pp. 159–164, 2015.

[175] H.-F. Liu, X.-W. Qi, L.-L. Ma, D.-K. Yao, and L. Wang, "Atorvastatin improves endothelial progenitor cell function and reduces pulmonary hypertension in patients with chronic pulmonary heart disease," *Experimental and Clinical Cardiology*, vol. 18, no. 1, pp. e40–e43, 2013.

[176] U. Landmesser, N. Engberding, F. H. Bahlmann et al., "Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase," *Circulation*, vol. 110, no. 14, pp. 1933–1939, 2004.

[177] M. A. Mughal, I. A. Alvi, I. A. Akhund, and A. K. Ansari, "The effects of aerobic exercise training on resting blood pressure in hypertensive patients," *Journal of the Pakistan Medical Association*, vol. 51, no. 6, pp. 222–226, 2001.

[178] S. A. M. Liu, J. Goodman, R. Nolan, S. Lacombe, and S. G. Thomas, "Blood pressure responses to acute and chronic exercise are related in prehypertension," *Medicine & Science in Sports & Exercise*, vol. 44, no. 9, pp. 1644–1652, 2012.

[179] A. Oberman, G. F. Fletcher, J. Lee et al., "Efficacy of high-intensity exercise training on left ventricular ejection fraction in men with coronary artery disease (The Training Level Comparison Study)," *The American Journal of Cardiology*, vol. 76, no. 10, pp. 643–647, 1995.

[180] N. Mathur and B. K. Pedersen, "Exercise as a mean to control low-grade systemic inflammation," *Mediators of Inflammation*, vol. 2008, Article ID 109502, 6 pages, 2008.

[181] B. Hoier and Y. Hellsten, "Exercise-induced capillary growth in human skeletal muscle and the dynamics of VEGF," *Microcirculation*, vol. 21, no. 4, pp. 301–314, 2014.

[182] J. B. Gillen, J. P. Little, Z. Punthakee, M. A. Tarnopolsky, M. C. Riddell, and M. J. Gibala, "Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycaemia in patients with type 2 diabetes," *Diabetes, Obesity and Metabolism*, vol. 14, no. 6, pp. 575–577, 2012.

[183] J. A. Babraj, N. B. J. Vollaard, C. Keast, F. M. Guppy, G. Cottrell, and J. A. Timmons, "Extremely short duration high intensity interval training substantially improves insulin action in young healthy males," *BMC Endocrine Disorders*, vol. 9, no. 1, article 3, 2009.

[184] V. Adams, K. Lenk, A. Linke et al., "Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise-induced ischemia," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 4, pp. 684–690, 2004.

[185] A. Aoki, M. Murata, T. Asano et al., "Prompt increases in retinol-binding protein 4 and endothelial progenitor cells during acute exercise load in diabetic subjects," *Endocrine Journal*, vol. 59, no. 12, pp. 1085–1091, 2012.

[186] M. R. Bonsignore, G. Morici, R. Riccioni et al., "Hemopoietic and angiogenetic progenitors in healthy athletes: different responses to endurance and maximal exercise," *Journal of Applied Physiology*, vol. 109, no. 1, pp. 60–67, 2010.

[187] E. Chang, J. Paterno, D. Duscher et al., "Exercise induces stromal cell-derived factor-1α-mediated release of endothelial progenitor cells with increased vasculogenic function," *Plastic and Reconstructive Surgery*, vol. 135, no. 2, pp. 340e–350e, 2015.

[188] E. Goussetis, A. Spiropoulos, M. Tsironi et al., "Spartathlon, a 246 kilometer foot race: effects of acute inflammation induced by prolonged exercise on circulating progenitor reparative cells," *Blood Cells, Molecules, and Diseases*, vol. 42, no. 3, pp. 294–299, 2009.

[189] N. T. Jenkins, S. Witkowski, E. E. Spangenburg, and J. M. Hagberg, "Effects of acute and chronic endurance exercise on intracellular nitric oxide in putative endothelial progenitor cells: role of NAPDH oxidase," *The American Journal of Physiology—Heart and Circulatory Physiology*, vol. 297, no. 5, pp. H1798–H1805, 2009.

[190] U. Laufs, A. Urhausen, N. Werner et al., "Running exercise of different duration and intensity: effect on endothelial progenitor cells in healthy subjects," *European Journal of Cardiovascular Prevention and Rehabilitation*, vol. 12, no. 4, pp. 407–414, 2005.

[191] S. Möbius-Winkler, T. Hilberg, K. Menzel et al., "Time-dependent mobilization of circulating progenitor cells during strenuous exercise in healthy individuals," *Journal of Applied Physiology*, vol. 107, no. 6, pp. 1943–1950, 2009.

[192] J. Rehman, J. Li, L. Parvathaneni et al., "Exercise acutely increases circulating endothelial progenitor cells and monocyte-/macrophage-derived angiogenic cells," *Journal of the American College of Cardiology*, vol. 43, no. 12, pp. 2314–2318, 2004.

[193] M. D. Ross, A. L. Wekesa, J. P. Phelan, and M. Harrison, "Resistance exercise increases endothelial progenitor cells and angiogenic factors," *Medicine and Science in Sports & Exercise*, vol. 46, no. 1, pp. 16–23, 2014.

[194] M. Sandri, E. B. Beck, V. Adams et al., "Maximal exercise, limb ischemia, and endothelial progenitor cells," *European Journal of Cardiovascular Prevention & Rehabilitation*, vol. 18, no. 1, pp. 55–64, 2011.

[195] M. Sandri, V. Adams, S. Gielen et al., "Effects of exercise and ischemia on mobilization and functional activation of blood-derived progenitor cells in patients with ischemic syndromes," *Circulation*, vol. 111, no. 25, pp. 3391–3399, 2005.

[196] G. Scalone, A. De Caterina, A. M. Leone et al., "Effect of exercise on circulating endothelial progenitor cells in microvascular angina," *Circulation Journal*, vol. 77, no. 7, pp. 1777–1782, 2013.

[197] R. Schier, R. El-Zein, A. Cortes et al., "Endothelial progenitor cell mobilization by preoperative exercise: a bone marrow response associated with postoperative outcome," *British Journal of Anaesthesia*, vol. 113, no. 4, pp. 652–660, 2014.

[198] R. G. Shaffer, S. Greene, A. Arshi et al., "Effect of acute exercise on endothelial progenitor cells in patients with peripheral arterial disease," *Vascular Medicine*, vol. 11, no. 4, pp. 219–226, 2006.

[199] E. M. Van Craenenbroeck, C. J. Vrints, S. E. Haine et al., "A maximal exercise bout increases the number of circulating CD34+/KDR+ endothelial progenitor cells in healthy subjects. Relation with lipid profile," *Journal of Applied Physiology*, vol. 104, no. 4, pp. 1006–1013, 2008.

[200] E. M. Van Craenenbroeck, L. Bruyndonckx, C. Van Berckelaer, V. Y. Hoymans, C. J. Vrints, and V. M. Conraads, "The effect of acute exercise on endothelial progenitor cells is attenuated in chronic heart failure," *European Journal of Applied Physiology*, vol. 111, no. 9, pp. 2375–2379, 2011.

[201] J.-S. Wang, M.-Y. Lee, H.-Y. Lien, and T.-P. Weng, "Hypoxic exercise training improves cardiac/muscular hemodynamics and is associated with modulated circulating progenitor cells in sedentary men," *International Journal of Cardiology*, vol. 170, no. 3, pp. 315–323, 2014.

[202] Z. Yang, J.-M. Wang, L. Chen, C.-F. Luo, A.-L. Tang, and J. Tao, "Acute exercise-induced nitric oxide production contributes to upregulation of circulating endothelial progenitor cells in healthy subjects," *Journal of Human Hypertension*, vol. 21, no. 6, pp. 452–460, 2007.

[203] J. L. Rummens, A. Daniëls, P. Dendale et al., "Suppressed increase in blood endothelial progenitor cell content as a result of single exhaustive exercise bout in male revascularised coronary artery disease patients," *Acta Clinica Belgica*, vol. 67, no. 4, pp. 262–269, 2012.

[204] V. Adams, A. Linke, F. Breuckmann et al., "Circulating progenitor cells decrease immediately after marathon race in advanced-age marathon runners," *European Journal of Cardiovascular Prevention & Rehabilitation*, vol. 15, no. 5, pp. 602–607, 2008.

[205] M. Okutsu, K. Ishii, K. J. Niu, and R. Nagatomi, "Cortisol-induced CXCR4 augmentation mobilizes T lymphocytes after acute physical stress," *The American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 288, no. 3, pp. R591–R599, 2005.

[206] O. A. Ajijola, C. Dong, E. E. Herderick, Q. Ma, P. J. Goldschmidt-Clermont, and Z. Yan, "Voluntary running suppresses proinflammatory cytokines and bone marrow endothelial progenitor cell levels in apolipoprotein-E-deficient mice," *Antioxidants & Redox Signaling*, vol. 11, no. 1, pp. 15–23, 2009.

[207] F. Cesari, R. Marcucci, A. M. Gori et al., "Impact of a cardiac rehabilitation program and inflammatory state on endothelial progenitor cells in acute coronary syndrome patients," *International Journal of Cardiology*, vol. 167, no. 5, pp. 1854–1859, 2013.

[208] F. Cesari, F. Sofi, R. Caporale et al., "Relationship between exercise capacity, endothelial progenitor cells and cytochemokines in patients undergoing cardiac rehabilitation," *Thrombosis and Haemostasis*, vol. 101, no. 3, pp. 521–526, 2009.

[209] J. K. Choi, K. M. Moon, S. Y. Jung et al., "Regular exercise training increases the number of endothelial progenitor cells and decreases homocysteine levels in healthy peripheral blood," *Korean Journal of Physiology and Pharmacology*, vol. 18, no. 2, pp. 163–168, 2014.

[210] T. Fernandes, J. S. Nakamuta, F. C. Magalhães et al., "Exercise training restores the endothelial progenitor cells number and function in hypertension: implications for angiogenesis," *Journal of Hypertension*, vol. 30, no. 11, pp. 2133–2143, 2012.

[211] J. M. Fernández, D. Rosado-Álvarez, M. E. Da Silva Grigoletto et al., "Moderate-to-high-intensity training and a hypocaloric Mediterranean diet enhance endothelial progenitor cells and fitness in subjects with the metabolic syndrome," *Clinical Science*, vol. 123, no. 6, pp. 361–373, 2012.

[212] C. Fiorito, M. L. Balestrieri, E. Crimi et al., "Effect of l-arginine on circulating endothelial progenitor cells and VEGF after moderate physical training in mice," *International Journal of Cardiology*, vol. 126, no. 3, pp. 421–423, 2008.

[213] L. Gatta, A. Armani, F. Iellamo et al., "Effects of a short-term exercise training on serum factors involved in ventricular remodelling in chronic heart failure patients," *International Journal of Cardiology*, vol. 155, no. 3, pp. 409–413, 2012.

[214] U. Laufs, N. Werner, A. Link et al., "Physical training increases endothelial progenitor cells, inhibits neointima formation, and

enhances angiogenesis," *Circulation*, vol. 109, no. 2, pp. 220–226, 2004.

[215] F. Manfredini, G. M. Rigolin, A. M. Malagoni et al., "Exercise training and endothelial progenitor cells in haemodialysis patients," *Journal of International Medical Research*, vol. 37, no. 2, pp. 534–540, 2009.

[216] P. Sarto, E. Balducci, G. Balconi et al., "Effects of exercise training on endothelial progenitor cells in patients with chronic heart failure," *Journal of Cardiac Failure*, vol. 13, no. 9, pp. 701–708, 2007.

[217] O. Schlager, A. Giurgea, O. Schuhfried et al., "Exercise training increases endothelial progenitor cells and decreases asymmetric dimethylarginine in peripheral arterial disease: a randomized controlled trial," *Atherosclerosis*, vol. 217, no. 1, pp. 240–248, 2011.

[218] K. Sonnenschein, T. Horváth, M. Mueller et al., "Exercise training improves in vivo endothelial repair capacity of early endothelial progenitor cells in subjects with metabolic syndrome," *European Journal of Cardiovascular Prevention & Rehabilitation*, vol. 18, no. 3, pp. 406–414, 2011.

[219] S. Steiner, A. Niessner, S. Ziegler et al., "Endurance training increases the number of endothelial progenitor cells in patients with cardiovascular risk and coronary artery disease," *Atherosclerosis*, vol. 181, no. 2, pp. 305–310, 2005.

[220] E. M. Van Craenenbroeck, V. Y. Hoymans, P. J. Beckers et al., "Exercise training improves function of circulating angiogenic cells in patients with chronic heart failure," *Basic Research in Cardiology*, vol. 105, no. 5, pp. 665–676, 2010.

[221] J. Zhang, X. Zhang, H. Li et al., "Hyperglycaemia exerts deleterious effects on late endothelial progenitor cell secretion actions," *Diabetes and Vascular Disease Research*, vol. 10, no. 1, pp. 49–56, 2013.

[222] S. Witkowski, M. M. Lockard, N. T. Jenkins, T. O. Obisesan, E. E. Spangenburg, and J. M. Hagberg, "Relationship between circulating progenitor cells, vascular function and oxidative stress with long-term training and short-term detraining in older men," *Clinical Science*, vol. 118, no. 4, pp. 303–311, 2010.

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Research Article

The Effects of Age and Latent Cytomegalovirus Infection on NK-Cell Phenotype and Exercise Responsiveness in Man

Austin B. Bigley, Guillaume Spielmann, Nadia Agha, and Richard J. Simpson

Laboratory of Integrated Physiology, Department of Health and Human Performance, University of Houston, 3855 Holman Street, Houston, TX 77204, USA

Correspondence should be addressed to Richard J. Simpson; rjsimpson@uh.edu

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The redeployment of NK-cells in response to an acute bout of exercise is thought to be an integral component of the "fight-or-flight" response, preparing the body for potential injury or infection. We showed previously that CMV seropositivity impairs the redeployment of NK-cells with exercise in the young. In the current study, we examined the effect of aging on the redeployment of NK-cells with exercise in the context of CMV. We show here that CMV blunts the exercise-induced redeployment of NK-cells in both younger (23–39 yrs) and older (50–64 yrs) subjects with older CMV^{neg} subjects showing the largest postexercise mobilization and 1 h postexercise egress of NK-cells. The blunted exercise response in CMV^{pos} individuals was associated with a decreased relative redeployment of the CD158a+ and CD57+ NK-cell subsets in younger and older individuals. In addition, we show that aging is associated with a CMV-independent increase in the proportion of NK-cells expressing the terminal differentiation marker CD57, while CMV is associated with an age-dependent decrease in the proportion of NK-cells expressing the inhibitory receptors KLRG1 (in the younger group) and CD158a (in the older group). Collectively, these data suggest that CMV may decrease NK-cell mediated immunosurveillance after exercise in both younger and older individuals.

1. Introduction

The rapid redeployment of NK-cells between the tissues and the peripheral circulation is an archetypal feature of the acute stress response. The response can be evoked using acute bouts of dynamic exercise [1, 2], laboratory-based psychological stress tasks [3], or beta-agonist (i.e., epinephrine) infusion [4] and is often considered to be an accurate representation of an organism's ability to mount an effective immune response during fight-or-flight scenarios when tissue injury and infection are likely to occur. Acute exercise is associated with increased plasma levels of stress hormones including the catecholamines epinephrine and norepinephrine [5], which interact with β-adrenergic receptors (β-AR) on the surface of lymphocytes. NK-cells express more β-AR than other lymphocytes [6] and, as a result, they are the most responsive lymphocyte subset to exercise [7, 8] and catecholamines [4, 9].

Cytomegalovirus (CMV) is a prevalent beta herpesvirus infecting 50–80% of the US population [10, 11]. We have shown that prior exposure to CMV profoundly impacts the redistribution of lymphocytes to an acute exercise bout. While those with CMV have an augmented redeployment of CD8+ T-cells [12, 13] and $\gamma\delta$ T-cells [14], NK-cell mobilization is dramatically impaired [15]. This blunted NK-cell response appears to be attributable to a CMV-induced accumulation of specific NK-cell subsets that have a lower expression of β2-AR and an impaired ability to produce cyclic AMP in response to *in vitro* stimulation with the β-agonist isoproterenol [16]. Moreover, those with CMV fail to exhibit exercise-induced enhancements in NK-cell function, indicating that CMV may compromise NK-cell mediated immunosurveillance after an acute bout of strenuous exercise [16].

In addition to infection history, aging is known to have a profound impact on the cellular response to acute stress and exercise [17]; however, studies investigating the effects

of aging on NK-cell exercise responsiveness are lacking [18]. While aging has been reported to have no effect on NK-cell mobilization with exercise [19, 20], it is known to increase the proportion of CD56dim/KIR+/CD57+ NK-cells [19, 21, 22], a subset we have previously shown to be preferentially mobilized by exercise [23]. In addition, several of the phenotypic hallmarks of aging overlap with those associated with latent CMV infection in the young including upregulation of CD57 [19, 24, 25] and downregulation of KLRG1 [15, 26]. Despite CMV prevalence increasing with age [11], previous studies have compared NK-cell responses between young and old exercisers without accounting for this confounding variable [19, 20]. We showed recently that CMV was associated with enhanced redeployment of CD8+ T-cells regardless of age [13], while, conversely, aging impairs the redeployment of $\gamma\delta$ T-cells independently of CMV [14]. However, no study to our knowledge has compared NK-cell responses to a single bout of exercise between different age groups while controlling for CMV serostatus. Given that CMV prevalence increases with age and many of the effects of CMV mirror those attributable to aging, it is important to resolve the effects of age and CMV infection on the frequency and exercise responsiveness of distinct NK-cell subsets.

The aim of this study was to determine if latent CMV infection blunts the redeployment of NK-cells to a single exercise bout in older individuals as it does in the young [15] and to delineate the effects of age and CMV on the redeployment of discrete NK-cell subsets. We show here that CMV has a potent blunting effect on exercise-induced NK-cell mobilization in both younger (23–39 yrs) and older (50–64 yrs) subjects with the greatest mobilization being seen in the CMV^{neg} older group. This blunting effect of CMV was most pronounced with the CD158a+ and CD57+ NK-cell subsets regardless of age.

2. Materials and Methods

2.1. Participants. 40 healthy adult males (age: 23–64 years) participated in this study. The exclusion criteria of this study required that participants avoid smoking, medication/supplements, or infection within 6 weeks of the experiment. Oral and written information regarding the risks and requirements of the study were provided, after which each participant signed an informed consent affidavit. Protocol approval was granted by CPHS at the University of Houston. Participant attributes and exercise data are provided in Table 1.

2.2. Exercise Protocols and Blood Sampling. Maximal oxygen uptake ($\dot{V}O_2$ max) was estimated using the Astrand [30] submaximal cycling exercise protocol as previously described [15]. The Adams and Beam equations [29] were used to estimate the $\dot{V}O_2$ max and maximum power of each participant.

Participants reported to the lab following an overnight fast within 2 weeks (minimum: 2 days) of the submaximal $\dot{V}O_2$ max test to complete a 30 min cycling protocol. A resting intravenous blood sample was collected in 6 mL Vacutainers containing either EDTA or serum gel (BD, Franklin Lakes, NJ, USA) prior to exercise. Participants then cycled for 30 min at 80% of max power and peripheral blood samples were collected again immediately after and 1 h after exercise. Serum was frozen at −80°C until analysis (in duplicate) for CMV IgG antibodies using commercially available ELISA kits (BioCheck, Foster City, CA, USA) and a 96-well microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.3. Flow Cytometry. PBMCs were separated from whole blood using Histopaque per the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). Aliquots of 1×10^6 isolated cells were incubated for 30 minutes with 50 μL of prediluted APC-conjugated anti-CD3, Alexa488-conjugated anti-KLRG1 [31] or FITC-conjugated anti-CD56, PerCP-Cy5.5-conjugated anti-CD8 or PerCP-eFluor710-conjugated anti-CD56, and PE-conjugated anti-CD57 or anti-CD158a monoclonal antibodies. All of the antibodies were purchased from eBioscience (San Diego, CA, USA) except for the anti-KLRG1 antibody that was generously provided by Dr. Hanspeter Pircher. Lymphocyte phenotype and cell count were assessed by 4-color flow cytometry using an Accuri C6 flow cytometer (Accuri, Ann Arbor, MI, USA) as previously described [15].

2.4. Statistical Analysis. SPSS version 22 (Chicago, IL, USA) was used for all statistical analyses. The effects of CMV and age on NK-cell phenotype, participant attributes, and exercise data were determined using a separate restricted maximum likelihood linear mixed model (LMM) including main effects for CMV serostatus and age, as well as an interaction term for CMV serostatus $*$ age. To determine the effects of CMV and age on the acute exercise response of NK-cell subsets, a LMM was built that included main effects for CMV serostatus, age, and exercise (before, after, and 1 h after), as well as interaction terms for CMV serostatus $*$ exercise and age $*$ exercise. The precise location of significant main effects was determined using Bonferroni post-hoc analysis. Independent sample t-tests were used to compare delta values (i.e., cells mobilized and egressed by exercise) relative to CMV serostatus and age. Statistical significance was assessed at $p < 0.05$.

3. Results

3.1. Age and CMV Have Distinct Effects on NK-Cell Phenotype. To determine the effects of age and CMV on NK-cell subsets, we evaluated NK-cell phenotype in the context of age and latent CMV infection (Figure 1). Aging increases the proportion of CD57+ NK-cells [$F(1, 180) = 6.551$, $p < 0.01$] and decreases the proportion of CD56-bright NK-cells [$F(1, 180) = 5.363$, $p < 0.05$]. The effects of age on CD57+ and CD56-bright NK-cells were CMV independent [$F(1, 180) = 0.37$, $p = 0.554$ and $F(1, 180) = 0.022$, $p = 0.882$, resp.]. While there was no overall effect of age on NK-cell CD158a expression [$F(1, 180) = 2.581$, $p = 0.111$], there was an interaction effect between CMV and age [$F(1, 180) = 3.91$, $p < 0.05$]. The proportion of CD158a+ NK-cells was lower in the CMV^{pos} older (50–64 yrs) group ($p < 0.05$). While there was no main effect of age on NK-cell KLRG1 [$F(1, 180)$

Table 1: Physical characteristics of the participants ($N_{CMV+} = 20$; $N_{CMV-} = 20$). Data are mean ± SD. CMV seronegative and CMV seropositive participants indicated by – and +, respectively. Statistical differences between younger (23–39 yrs) and older (50–64 yrs) subjects are indicated by $^{\#}p < 0.05$. CMV serostatus did not affect any of the physical characteristics ($p > 0.05$).

Characteristics	Younger (–) [$N = 12$]	Younger (+) [$N = 12$]	Older (–) [$N = 8$]	Older (+) [$N = 8$]	One-way ANOVA F statistic (p value)	
					CMV	Age
Age (years)	30.8 ± 6.4	30.0 ± 6.0	55.6 ± 3.6$^{\#}$	57.1 ± 4.1$^{\#}$	<0.1 (0.88)	163.1 (<0.001)
Height (cm)	179.4 ± 7.1	177.9 ± 5.4	178.2 ± 8.6	177.2 ± 6.3	1.3 (0.26)	0.1 (0.72)
Mass (kg)	81.2 ± 12.8	82.8 ± 12.1	79.6 ± 8.1	79.8 ± 10.2	<0.1 (0.98)	<0.1 (0.84)
BMI ($kg{\cdot}m^{-2}$)	25.1 ± 3.2	25.7 ± 4.7	24.8 ± 2.1	25.8 ± 2.7	0.5 (0.45)	<0.1 (0.90)
Maximum power (W)	241.3 ± 76.5	226.2 ± 57.0	235.7 ± 65.6	225.8 ± 38.8	0.4 (0.53)	<0.1 (0.88)
VO_{2max} ($mL{\cdot}kg^{-1}{\cdot}min^{-1}$)	39.6 ± 13.0	36.7 ± 6.3	40.7 ± 17.4	40.3 ± 7.5	0.4 (0.49)	<0.1 (0.90)
Maximum heart rate* (bpm)	187.7 ± 6.1	187.8 ± 5.8	164.4 ± 3.6	164.9 ± 3.7	<0.1 (0.87)	163.4 (<0.001)
Physical activity rating[a] (0–7)	5.0 ± 2.2	5.4 ± 2.1	6.3 ± 1.0	5.6 ± 1.9	<0.1 (0.90)	3.2 (0.09)
Age-adjusted fitness score[b] (1–5)	3.6 ± 1.8	3.1 ± 1.1	4.1 ± 1.2	3.7 ± 1.0	<0.1 (0.83)	2.2 (0.14)
Exercise measures						
Mean power (W)	183.6 ± 63.5	175.8 ± 61.1	181.3 ± 38.5	172.4 ± 29.7	0.2 (0.62)	<0.1 (0.87)
Mean power (% max)	76.3 ± 11.9	77.5 ± 13.8	77.9 ± 6.2	76.7 ± 7.0	<0.1 (0.99)	<0.1 (0.92)
Mean Heart rate (bpm)	158.7 ± 10.3	159.6 ± 7.5	151.0 ± 7.8$^{\#}$	147.5 ± 8.1$^{\#}$	0.2 (0.64)	13.1 (0.001)
Mean heart rate (% max)*	85.4 ± 5.4	84.5 ± 2.7	91.7 ± 4.9$^{\#}$	89.1 ± 3.7$^{\#}$	1.8 (0.20)	14.6 (<0.001)

*Maximum heart rate estimated by the equation: $191.5 - (0.007 \times age^2)$ [27].
[a]Physical Activity Rating (PA-R) calculated from Jackson Questionnaire [28].
[b]Age-adjusted Fitness Score calculated from Adams and Beam equations [29].

= 0.154, $p = 0.695$] or CD8 expression [$F(1, 180) = 1.904$, $p = 0.169$], the proportion of KLRG1+ NK-cells was greater in the CMV^{neg} younger (23–39 yrs) group ($p < 0.05$) and the proportion of CD8+ NK-cells was greater in the CMV^{neg} older group ($p < 0.05$).

3.2. Latent CMV Infection Impairs the Exercise-Induced Mobilization of NK-Cells in Both Younger and Older Adults. The effects of age and CMV serostatus on the exercise response of total NK-cells and NK-cell subsets are shown in Table 2. The number of total NK-cells and all NK-cell subsets was increased immediately after exercise compared to baseline and 1 h after exercise ($p < 0.001$). There was a main effect of CMV serostatus on total NK-cell number [$F(1, 168) = 5.652$, $p < 0.05$] that was independent of age [$F(1, 168) = 1.323$, $p = 0.252$]. The main effect of CMV serostatus was the result of fewer NK-cells immediately after exercise ($p < 0.05$). There was no main effect of age on total NK-cell number [$F(1, 168) = 2.617$, $p = 0.108$]; however, the postexercise NK-cell count was greater in older relative to younger CMV^{neg} subjects ($p < 0.05$).

The exercise responsiveness of NK-cells based on CMV serostatus and age is described in Figure 2(a). CMV seropositivity was associated with a lower exercise-induced redeployment of NK-cells [$F(2, 168) = 4.664$, $p < 0.05$] that was independent of age [$F(2, 168) = 1.037$, $p = 0.357$]. Specifically, the mobilization and egress of NK-cells were greater in CMV^{neg} individuals regardless of age ($p < 0.05$) as seen in Figure 2(b). Age did not affect the redeployment of NK-cells with exercise [$F(2, 168) = 0.587$, $p = 0.557$].

Aging was associated with an increased percentage of NK-cells within the lymphocyte pool [$F(1, 168) = 5.123$, $p = 0.025$] that was dependent on CMV serostatus [$F(1, 168) = 12.777$, $p < 0.001$]. Specifically, the proportion of NK-cells was elevated in older subjects that were CMV^{neg} ($p < 0.05$) but not in those that were infected with CMV ($p > 0.05$). As with cell number, CMV seropositivity was associated with a lower proportional increase in NK-cells after exercise [$F(2, 168) = 3.221$, $p < 0.05$] that was independent of age [$F(2, 168) = 1.258$, $p = 0.287$]. Representative flow cytometry dot-plots illustrating the age-independent blunting of NK-cell exercise responsiveness in those infected with CMV are shown in Figure 2(c).

3.3. Latent CMV Infection Decreases the Exercise Responsiveness of CD57+ and CD158a+ NK-Cells Independently of Age. To assess the effects of age and CMV on the redeployment of NK-cell subsets with exercise, we evaluated exercise-induced changes in the proportion of NK-cell subsets in relation to age and latent CMV infection (Figure 3(a)). The proportion of CD57+ and CD158a+ NK-cells is increased immediately after exercise ($p < 0.05$). The exercise effects on NK-cell CD57 and CD158a expression were independent of CMV [$F(2, 180) = 0.016$, $p = 0.984$ and $F(2, 180) = 0.075$, $p = 0.927$, resp.] and age [$F(2, 180) = 0.213$, $p = 0.808$ and $F(2, 180) = 0.312$, $p = 0.732$, resp.]. The mobilization and egress of CD57+

Table 2: Exercise-induced changes in NK-cell subset numbers in healthy adult males ($N_{CMV+} = 20$; $N_{CMV-} = 20$) contrasted by CMV serostatus and age. CMV^{pos} and CMV^{neg} participants are denoted by + and −, respectively. Main and interaction effects for CMV, age, and time are reported with significance connoted by $^*(p < 0.05)$. Statistical differences from pre- and 1 h post-values are described by # and ∧, respectively ($p < 0.05$). Data are mean ± SD.

NK-Cell subsets	CMV status	Pre-values	Post-values	1 h post-values	Main effects F statistic (p value)			Interactions F statistic (p value)			
					Time	Age	CMV	Time × Age	Time × CMV	Age × CMV	Time × Age × CMV
Total NK-cells/μL			#∧	#	157.7* (<0.001)	2.6 (0.108)	5.7* (0.019)	0.6 (0.557)	4.7* (0.019)	1.3 (0.252)	1.0 (0.357)
Younger	−	166 ± 96	785 ± 337	130 ± 117							
(23–39 yrs)	+	181 ± 97	595 ± 360	128 ± 84							
Older	−	223 ± 56	987 ± 182	142 ± 57							
(50–64 yrs)	+	189 ± 85	666 ± 242	153 ± 69							
CD56dim (cells/μL)			#∧	#	157.1* (<0.001)	2.8 (0.085)	5.6* (0.020)	0.7 (0.518)	4.7* (0.018)	1.7 (0.197)	1.1 (0.333)
Younger	−	147 ± 91	738 ± 325	106 ± 100							
(23–39 yrs)	+	162 ± 94	556 ± 357	112 ± 82							
Older	−	205 ± 58	942 ± 184	125 ± 57							
(50–64 yrs)	+	176 ± 79	631 ± 217	135 ± 54							
CD56 bright (cells/μL)			#∧		25.2* (<0.001)	1.1 (0.291)	2.5 (0.114)	<0.1 (0.999)	0.4 (0.687)	<0.1 (0.834)	0.5 (0.636)
Younger	−	19 ± 12	47 ± 27	24 ± 22							
(23–39 yrs)	+	19 ± 9	39 ± 15	16 ± 7							
Older	−	18 ± 9	45 ± 23	17 ± 6							
(50–64 yrs)	+	13 ± 10	35 ± 31	18 ± 15							
KLRG1+ (cells/μL)			#∧		72.6* (<0.001)	0.1 (0.773)	2.1 (0.153)	0.1 (0.929)	1.1 (0.335)	0.1 (0.788)	0.1 (0.948)
Younger	−	98 ± 76	493 ± 240	80 ± 73							
(23–39 yrs)	+	84 ± 70	334 ± 284	56 ± 64							
Older	−	111 ± 45	481 ± 149	78 ± 13							
(50–64 yrs)	+	95 ± 64	398 ± 198	69 ± 44							
CD57+ (cells/μL)			#∧		98.5* (<0.001)	5.3* (0.023)	5.2* (0.024)	1.7 (0.196)	2.5 (0.084)	2.7 (0.105)	1.0 (0.360)
Younger	−	63 ± 39	335 ± 190	53 ± 49							
(23–39 yrs)	+	74 ± 48	310 ± 184	50 ± 32							
Older	−	125 ± 29	578 ± 146	87 ± 29							
(50–64 yrs)	+	110 ± 46	453 ± 109	80 ± 29							
CD158a+ (cells/μL)			#∧		50.7* (<0.001)	0.9 (0.349)	2.7 (0.103)	0.3 (0.717)	1.3 (0.284)	0.2 (0.698)	<0.1 (0.980)
Younger	−	40 ± 25	228 ± 191	31 ± 34							
(23–39 yrs)	+	40 ± 20	179 ± 87	28 ± 19							
Older	−	51 ± 37	277 ± 91	32 ± 28							
(50-64 yrs)	+	25 ± 16	119 ± 97	17 ± 15							
CD8+ (cells/μL)			#∧		79.4* (<0.001)	8.4* (0.004)	1.2 (0.269)	2.7 (0.069)	1.7 (0.193)	4.8* (0.031)	3.0 (0.052)
Younger	−	51 ± 35	184 ± 126	45 ± 46							
(23–39 yrs)	+	61 ± 37	210 ± 94	42 ± 28							
Older	−	94 ± 36	406 ± 159	62 ± 21							
(50–64 yrs)	+	62 ± 43	200 ± 75	39 ± 22							

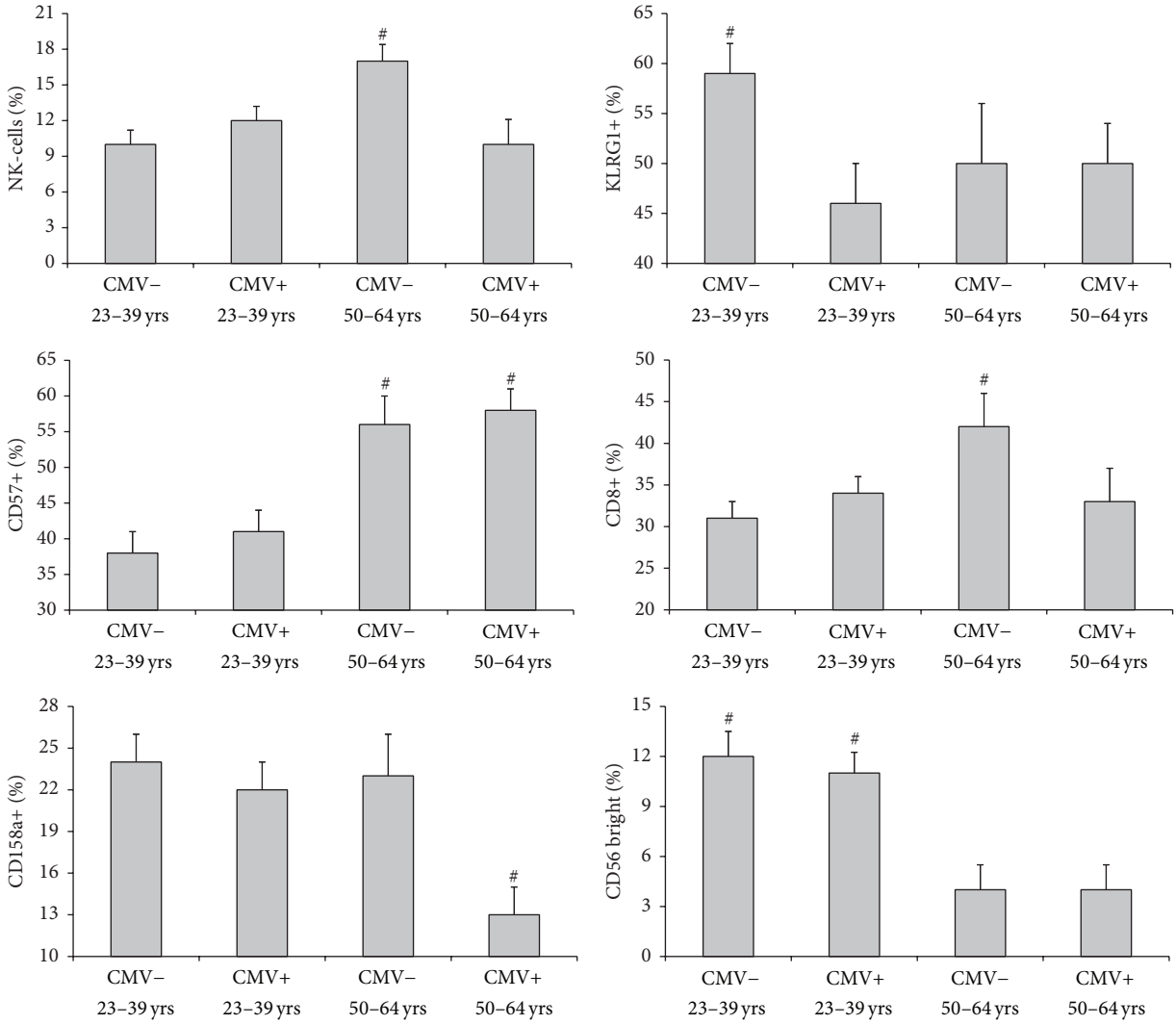


Figure 1: The effects of latent CMV infection and age on the proportion of total NK-cells (% of lymphocytes) and NK-cell subsets (% of NK-cells). Values are mean ± SE. Significance is connoted by $^{\#}p < 0.05$.

and CD158a+ NK-cells were greater in CMV-seronegative subjects relative to those infected with CMV ($p < 0.05$). In addition, the proportion of CD56-bright NK-cells was increased 1 h after exercise ($p < 0.05$) independently of CMV [$F(2, 180) = 0.059$, $p = 0.942$] and age [$F(2, 180) = 0.016$, $p = 0.984$]. Exercise had no effect on NK-cell KLRG1 or CD8 expression [$F(2, 180) = 0.404$, $p = 0.668$ and $F(2, 180) = 0.018$, $p = 0.982$, resp.]. In addition, CMV serostatus did not affect the mobilization or egress of CD56-bright, KLRG1+, or CD8+ NK-cells ($p > 0.05$). Representative flow cytometry dot-plots for the coexpression of KLRG1 and CD57 in relation to exercise are shown in Figure 3(b).

4. Conclusions

This is the first study to examine the effects of aging and latent CMV infection on NK-cell redeployment in response to a single bout of intensity-controlled exercise. We report that latent CMV infection is associated with a blunted exercise-induced redeployment of NK-cells in both younger (23–39 yrs) and older (50–64 yrs) subjects with older CMV^{neg} subjects showing the greatest postexercise mobilization and 1 h postexercise egress of NK-cells. This blunted exercise response in CMV^{pos} individuals was associated with a decreased relative redeployment of the CD158a+ and CD57+ NK-cell subsets in both younger and older individuals. In addition, we show for the first time that the previously reported age-associated increase in the proportion of CD57+ NK-cells is independent of CMV, while the proportion of CD8+ NK-cells and the percentage of NK-cells in the lymphocyte pool are increased in older CMV^{neg} individuals only. Further, CMV is associated with an age-dependent decrease in the proportion of NK-cells expressing the inhibitory receptors KLRG1 (lower in younger CMV^{pos}) and CD158a (lower in older CMV^{pos}).

The redeployment of cytotoxic lymphocytes (including NK-cells) in response to an acute bout of exercise is thought

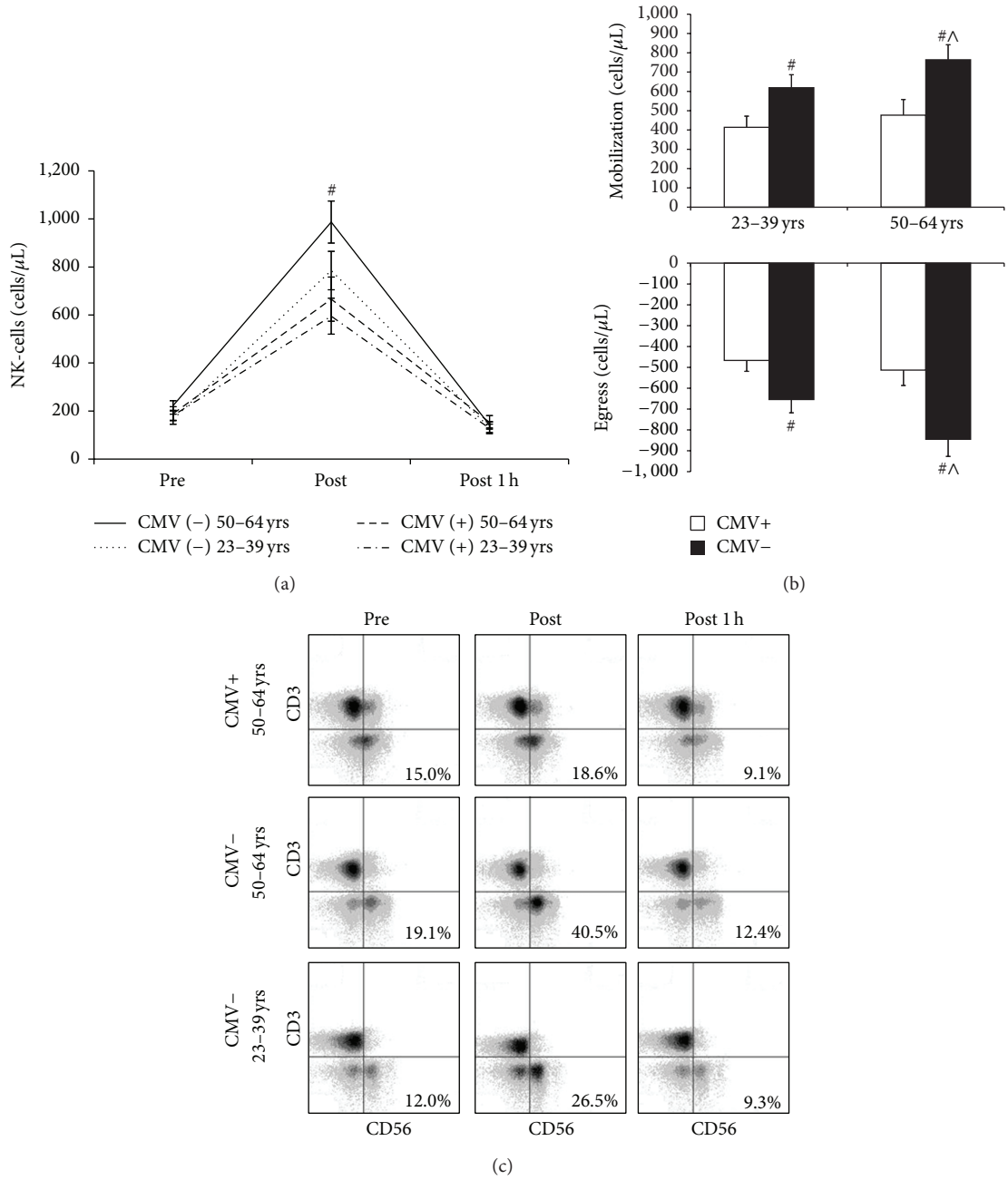
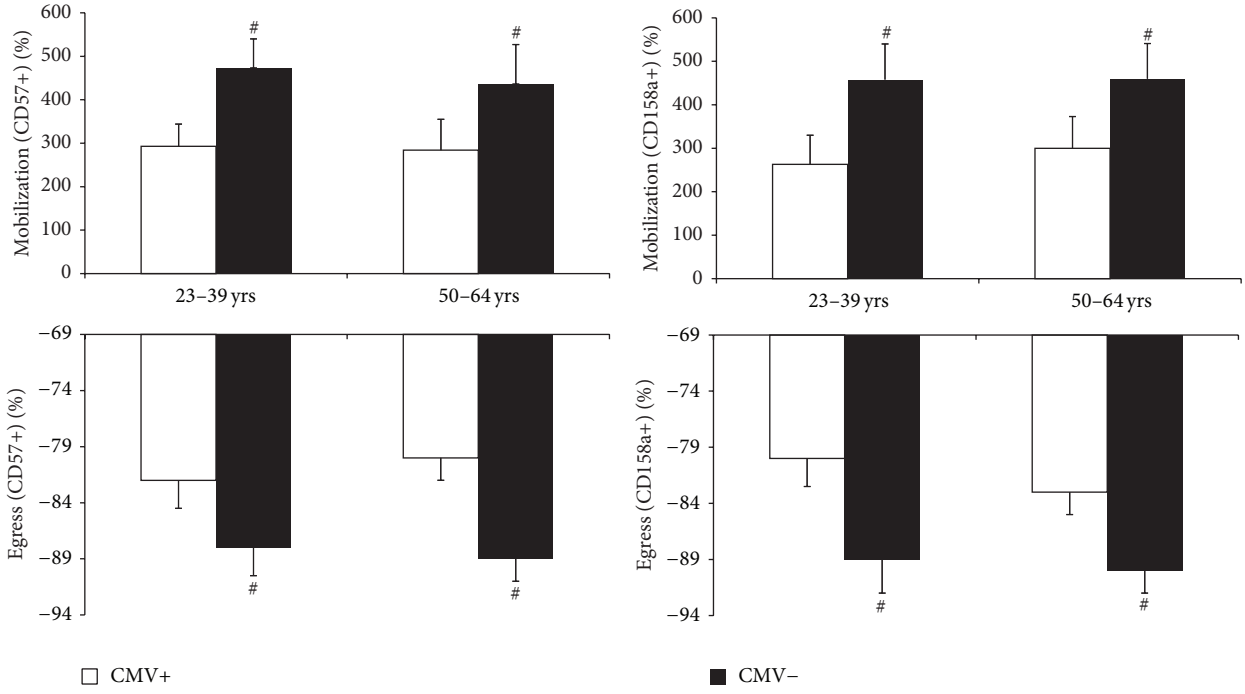
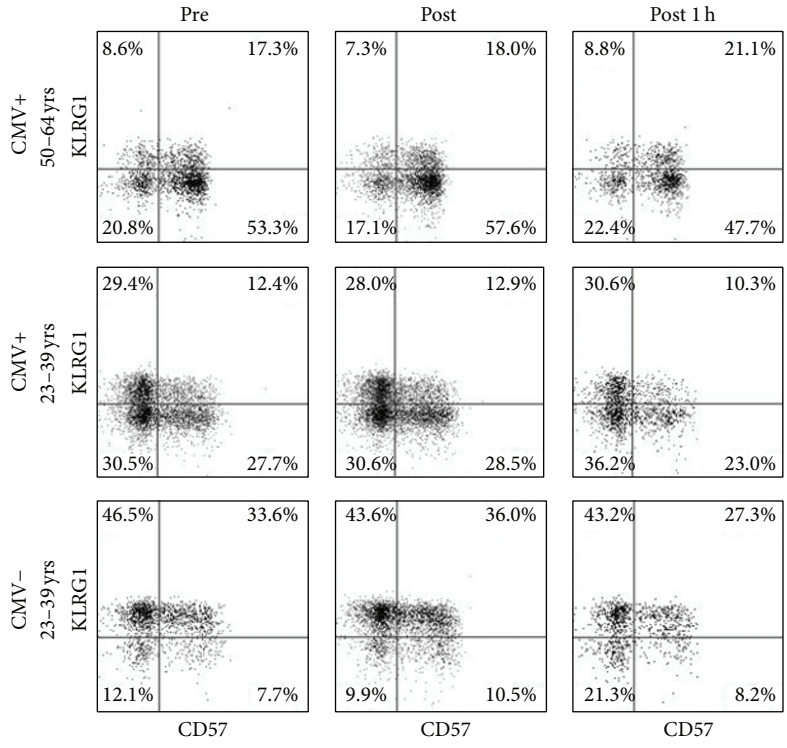


Figure 2: Latent CMV infection impairs the exercise-induced mobilization of NK-cells in both younger and older adults. (a) shows the effect of exercise at 80% of estimated maximum power on NK-cell count based on latent CMV infection and age. (b) shows the effect of latent CMV infection and age on the mobilization and egress of NK-cells in response to an acute bout of exercise at 80% of estimated maximum power. Values are mean ± SE. Differences based on CMV serostatus and age are indicated by $^{\#}p < 0.05$ and $^{\wedge}p < 0.05$, respectively. (c) displays representative flow cytometry dot-plots for the redeployment of NK-cells with exercise relative to CMV serostatus and age.

to be an integral part of the "fight-or-flight" response, preparing the body for potential injury or infection [3]. We show here that CMV seropositivity is associated with a blunted redeployment of NK-cells in older subjects and that increased age is associated with a greater redeployment of NK-cells in CMV^{neg} individuals. This builds on our previous finding that latent CMV infection is associated with a blunted exercise-induced redeployment of NK-cells in the young [15]. Alternatively, it has been reported that the mobilization of CD8+ cytotoxic T-cells is greater in CMV^{pos} individuals [12] and we have shown that this CMV effect is particularly pronounced in the old [13]. Considering that there is no difference in lymphocyte mobilization between CMV^{pos} and CMV^{neg} individuals [12, 13], it appears that the effects of CMV on redeployment of cytotoxic CD8+ T-cells and NK-cells largely offset each other. Thus, it could



(a)



(b)

Figure 3: Latent CMV infection decreases the exercise responsiveness of CD57+ and CD158a+ NK-cells independently of age. (a) shows the effects of CMV and age on the redeployment of CD57+ and CD158a+ NK-cells in response to an acute bout of exercise at 80% of estimated maximum power. Values are mean ± SE. Differences based on CMV serostatus are connoted by $^{\#}p < 0.05$. (b) displays representative flow cytometry dot-plots for the coexpression of KLRG1 and CD57 before and after exercise based on latent CMV infection and age.

be that postexercise immunosurveillance is tilted towards T-cell-mediated immunity in CMV^{pos} individuals and NK-cell mediated immunity in CMV^{neg} individuals with this contrast being most evident in older individuals. Given the decline in T-cell function with age [32] and the accumulation of senescent T-cells in those with CMV [33], this suggests a general decline in postexercise immunosurveillance in older CMV^{pos} individuals.

The redeployment of individual NK-cell subsets with exercise is nonuniform. We have previously reported that NK-cells expressing inhibitory KIR (such as CD158a) and the terminal differentiation marker CD57 are preferentially mobilized by exercise [23]. In the current study, we report that the mobilization of CD158a+ and CD57+ NK-cells with exercise is reduced in CMV^{pos} individuals regardless of age. We have shown earlier that CMV impairs NK-cell mobilization in response to high intensity exercise through downregulation of β2-AR expression on CD57+ NK-cells and impaired β-AR signaling in younger CMV^{pos} individuals [16]. This mechanism likely applies to older subjects as well given the similar impairment in CD57+ NK-cell exercise responsiveness between younger and older CMV^{pos} subjects. The decreased exercise-induced redeployment of CD57+ NK-cells in CMV^{pos} individuals is likely to have functional implications as CD57+ NK-cells have high cytotoxic functions, but greatly reduced proliferative capacity [34, 35]. Due to their high expression of differentiation markers [36] and their poor cytokine-driven proliferation, CD57+ NK-cells are considered to be terminally differentiated [34].

We show here that CMV and age combine to influence NK-cell phenotype in many interesting ways that have often been overlooked in the literature due to failure to recruit older CMV^{neg} individuals. For example, it has been reported in multiple studies that the proportion of CD57+ NK-cells is increased in the elderly [19, 37, 38]; however, it has been reported by Campos et al. that this aging effect is actually a CMV effect with CMV^{pos} subjects having a higher proportion of CD57+ NK-cells regardless of age [24]. The conclusions of Campos et al. are limited, however, by the lack of CMV^{neg} elderly in their cohort [24]. Our study includes older CMV^{neg} individuals and contradicts the findings of Campos et al. [24] as we show a CMV-independent increase in the proportion of CD57+ NK-cells with aging. These findings are consistent with the CMV-independent increase in the CD56dim : CD56 bright ratio with age shown here and elsewhere [21], which suggests that the accumulation of highly differentiated NK-cells is attributable to aging independently of latent CMV infection. The increased proportion of CD56dim CD57+ NK-cells in the older group likely contributes to the increased exercise-induced redeployment of NK-cells in older relative to younger CMV^{neg} individuals. One of the limitations of this study is that we did not measure CD16 expression on NK-cells. CD16 is a marker of NK-cell differentiation and it is functionally important as it plays a critical role in NK-cell mediated antibody-dependent cytotoxicity [39]. We were unable to include CD16 as a marker of differentiation because we were limited by the constraints of 4-color flow cytometry.

We report a CMV-dependent decrease in the proportion of CD158a+ NK-cells with age. There was no overall difference in the proportion of CD158a+ NK-cells between younger and older subjects; however, the proportion of CD158a+ NK-cells was decreased in CMV^{pos} older subjects. Multiple studies have reported no effect of aging on inhibitory KIR expression [38, 40, 41]; however, one study reported an increase in inhibitory KIR expression with age [22]. None of these earlier studies investigated the relationship between these aging effects and CMV infection. We also show that the proportion of KLRG1+ NK-cells is decreased in younger CMV^{pos} individuals, but there is no CMV effect in the older group and no overall aging effect either. Our current data contradict a previous report by Hayhoe et al. that aging is associated with a marked decrease in the proportion of KLRG1+ NK-cells [26]. The discrepancy between our findings and those of Hayhoe et al. [26] are likely due to different definitions of young and old between the two studies. Hayhoe et al. compared individuals older and younger than 60 years [26], while we compared individuals aged 23–39 years to individuals aged 50–64 years. In our study, we investigated aging in preelderly (less than 65 years old), otherwise healthy individuals (presymptomatic for any age-related diseases). Future studies should determine if our results regarding the effects of aging and CMV on NK-cell phenotype and exercise responsiveness apply to females and individuals over the age of 65 as well.

In addition, we show for the first time that the previously reported increase in NK-cell proportion with age [22, 24] is CMV-dependent. Specifically, the proportion of NK-cells in the peripheral lymphocyte pool is elevated in CMV^{neg} older subjects, but not older subjects with CMV. The proportion of CD8+ NK-cells is also elevated in CMV^{neg} older subjects only, which suggests a possible increase in NK-cell cytotoxicity as CD8 expression has been mechanistically linked to increased NK-cell activity [42]. On the other hand, we have reported increased baseline NK-cell activity and impaired functional responses to exercise in younger CMV^{pos} individuals [16]. Thus, future studies are needed to determine the combined effects of CMV and aging on NK-cell function before and after exercise.

In summary, latent CMV infection was associated with a marked reduction in the mobilization of NK-cells in response to exercise in both younger and older adult males with the greatest mobilization being seen in CMV^{neg} older subjects. We also show that age was associated with a CMV-independent increase in the proportion of terminally differentiated CD57+ NK-cells and that CMV was associated with an age-dependent decrease in the proportion of NK-cells expressing the inhibitory receptors KLRG1 (lower in younger group) and CD158a (lower in older group). We conclude that latent CMV infection may compromise NK-cell mediated immunosurveillance following an acute stress response in both younger and older males.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] R. J. Shephard and P. N. Shek, "Effects of exercise and training on natural killer cell counts and cytolytic activity," *Sports Medicine*, vol. 28, no. 3, pp. 177–195, 1999.

[2] A. Crisafulli, F. Tocco, F. Melis, R. Milia, and A. Concu, "Natural killer cells responsiveness to physical exercise: a brief review," *Open Journal of Immunology*, vol. 3, no. 4, pp. 190–200, 2013.

[3] J. A. Bosch, G. G. Berntson, J. T. Cacioppo, and P. T. Marucha, "Differential mobilization of functionally distinct natural killer subsets during acute psychologic stress," *Psychosomatic Medicine*, vol. 67, no. 3, pp. 366–375, 2005.

[4] M. Schedlowski, W. Hosch, R. Oberbeck et al., "Catecholamines modulate human NK cell circulation and function via spleen-independent beta 2-adrenergic mechanisms," *The Journal of Immunology*, vol. 156, no. 1, pp. 93–99, 1996.

[5] J. E. Dimsdale and J. Moss, "Plasma catecholamines in stress and exercise," *Journal of the American Medical Association*, vol. 243, no. 4, pp. 340–342, 1980.

[6] L. J. H. van Tits, M. C. Michel, H. Grosse-Wilde et al., "Catecholamines increase lymphocyte beta2-adrenergic receptors via a beta2-adrenergic, spleen-dependent process," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 258, no. 1, pp. E191–E202, 1990.

[7] M. Kappel, N. Tvede, H. Galbo et al., "Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine," *Journal of Applied Physiology*, vol. 70, no. 6, pp. 2530–2534, 1991.

[8] N. Tvede, M. Kappel, K. Klarhund et al., "Evidence that the effect of bicycle exercise on blood mononuclear cell proliferative responses and subsets is mediated by epinephrine," *International Journal of Sports Medicine*, vol. 15, no. 2, pp. 100–104, 1994.

[9] B. Crary, S. L. Hauser, M. Borysenko et al., "Epinephrine-induced changes in the distribution of lymphocyte subsets in peripheral blood of humans," *Journal of Immunology*, vol. 131, no. 3, pp. 1178–1181, 1983.

[10] S. L. Bate, S. C. Dollard, and M. J. Cannon, "Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988–2004," *Clinical Infectious Diseases*, vol. 50, no. 11, pp. 1439–1447, 2010.

[11] S. C. Dollard, S. A. S. Staras, M. M. Amin, D. S. Schmid, and M. J. Cannon, "National prevalence estimates for cytomegalovirus IgM and IgG avidity and association between high IgM antibody titer and low IgG avidity," *Clinical and Vaccine Immunology*, vol. 18, no. 11, pp. 1895–1899, 2011.

[12] J. E. Turner, S. Aldred, O. C. Witard, M. T. Drayson, P. M. Moss, and J. A. Bosch, "Latent cytomegalovirus infection amplifies CD8 T-lymphocyte mobilisation and egress in response to exercise," *Brain, Behavior, and Immunity*, vol. 24, no. 8, pp. 1362–1370, 2010.

[13] G. Spielmann, C. M. Bollard, A. B. Bigley et al., "The effects of age and latent cytomegalovirus infection on the redeployment of $CD8^+$ T cell subsets in response to acute exercise in humans," *Brain, Behavior, and Immunity*, vol. 39, pp. 142–151, 2013.

[14] M. Pistillo, A. B. Bigley, G. Spielmann et al., "The effects of age and viral serology on $\gamma\delta$ T-cell numbers and exercise responsiveness in humans," *Cellular Immunology*, vol. 284, no. 1-2, pp. 91–97, 2013.

[15] A. B. Bigley, T. W. Lowder, G. Spielmann et al., "NK-cells have an impaired response to acute exercise and a lower expression of the inhibitory receptors KLRG1 and CD158a in humans with latent cytomegalovirus infection," *Brain, Behavior, and Immunity*, vol. 26, no. 1, pp. 177–186, 2012.

[16] A. B. Bigley, K. Rezvani, M. Pistillo et al., "Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target cells. Part II. Impact of latent cytomegalovirus infection and catecholamine sensitivity," *Brain, Behavior, and Immunity*, 2015.

[17] A. C. Phillips, V. E. Burns, and J. M. Lord, "Stress and exercise: getting the balance right for aging immunity," *Exercise and Sport Sciences Reviews*, vol. 35, no. 1, pp. 35–39, 2007.

[18] D. A. Haaland, T. F. Sabljic, D. A. Baribeau, I. M. Mukovozov, and L. E. Hart, "Is regular exercise a friend or foe of the aging immune system? A systematic review," *Clinical Journal of Sport Medicine*, vol. 18, no. 6, pp. 539–548, 2008.

[19] R. J. Simpson, C. Cosgrove, L. A. Ingram et al., "Senescent T-lymphocytes are mobilised into the peripheral blood compartment in young and older humans after exhaustive exercise," *Brain, Behavior, and Immunity*, vol. 22, no. 4, pp. 544–551, 2008.

[20] J. A. Woods, J. K. Evans, B. W. Wolters, M. A. Ceddia, and E. McAuley, "Effects of maximal exercise on natural killer (NK) cell cytotoxicity and responsiveness to interferon-alpha in the young and old," *Journals of Gerontology—Series A: Biological Sciences and Medical Sciences*, vol. 53, no. 6, pp. B430–B437, 1998.

[21] S. Chidrawar, N. Khan, W. Wei et al., "Cytomegalovirus-seropositivity has a profound influence on the magnitude of major lymphoid subsets within healthy individuals," *Clinical and Experimental Immunology*, vol. 155, no. 3, pp. 423–432, 2009.

[22] C. T. Lutz, M. B. Moore, S. Bradley, B. J. Shelton, and S. K. Lutgendorf, "Reciprocal age related change in natural killer cell receptors for MHC class I," *Mechanisms of Ageing and Development*, vol. 126, no. 6-7, pp. 722–731, 2005.

[23] A. B. Bigley, K. Rezvani, C. Chew et al., "Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target cells," *Brain, Behavior, and Immunity*, vol. 39, pp. 160–171, 2014.

[24] C. Campos, A. Pera, B. Sanchez-Correa et al., "Effect of age and CMV on NK cell subpopulations," *Experimental Gerontology*, vol. 54, pp. 130–137, 2014.

[25] S. Lopez-Vergès, J. M. Milush, B. S. Schwartz et al., "Expansion of a unique $CD57^+NKG2C^{hi}$ natural killer cell subset during acute human cytomegalovirus infection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 36, pp. 14725–14732, 2011.

[26] R. P. G. Hayhoe, S. M. Henson, A. N. Akbar, and D. B. Palmer, "Variation of human natural killer cell phenotypes with age: identification of a unique KLRG1-negative subset," *Human Immunology*, vol. 71, no. 7, pp. 676–681, 2010.

[27] R. L. Gellish, B. R. Goslin, R. E. Olson, A. McDonald, G. D. Russi, and V. K. Moudgil, "Longitudinal modeling of the relationship between age and maximal heart rate," *Medicine and Science in Sports and Exercise*, vol. 39, no. 5, pp. 822–829, 2007.

[28] A. S. Jackson, S. N. Blair, M. T. Mahar, L. T. Wier, R. M. Ross, and J. E. Stuteville, "Prediction of functional aerobic capacity without exercise testing," *Medicine & Science in Sports & Exercise*, vol. 22, no. 6, pp. 863–870, 1990.

[29] G. M. Adams and W. C. Beam, *Exercise Physiology: Laboratory Manual*, McGraw-Hill, Boston, Mass, USA, 1998.

[30] I. Astrand, "Aerobic work capacity in men and women with special reference to age," *Acta Physiologica Scandinavica*, vol. 49, no. 1, pp. 1–92, 1960.

[31] I. Marcolino, G. K. Przybylski, M. Koschella et al., "Frequent expression of the natural killer cell receptor KLRG1 in human cord blood T cells: Correlation with replicative history," *European Journal of Immunology*, vol. 34, no. 10, pp. 2672–2680, 2004.

[32] T. Fülöp, A. Larbi, and G. Pawelec, "Human T cell aging and the impact of persistent viral infections," *Frontiers in Immunology*, vol. 4, article 271, Article ID Article 271, 2013.

[33] G. Pawelec, E. Derhovanessian, A. Larbi, J. Strindhall, and A. Wikby, "Cytomegalovirus and human immunosenescence," *Reviews in Medical Virology*, vol. 19, no. 1, pp. 47–56, 2009.

[34] S. Lopez-Vergès, J. M. Milush, S. Pandey et al., "CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset," *Blood*, vol. 116, no. 19, pp. 3865–3874, 2010.

[35] N. K. Björkström, P. Riese, F. Heuts et al., "Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education," *Blood*, vol. 116, no. 19, pp. 3853–3864, 2010.

[36] V. Béziat, B. Descours, C. Parizot, P. Debré, and V. Vieillard, "NK cell terminal differentiation: correlated stepwise decrease of NKG2A and acquisition of KIRs," *PLoS ONE*, vol. 5, no. 8, Article ID e11966, 2010.

[37] F. Borrego, M. C. Alonso, M. D. Galiani et al., "NK phenotypic markers and IL2 response in NK cells from elderly people," *Experimental Gerontology*, vol. 34, no. 2, pp. 253–265, 1999.

[38] M. Le Garff-Tavernier, V. Béziat, J. Decocq et al., "Human NK cells display major phenotypic and functional changes over the life span," *Aging Cell*, vol. 9, no. 4, pp. 527–535, 2010.

[39] B. Perussia, G. Trinchieri, A. Jackson et al., "The Fc receptor for IgG on human natural killer cells: phenotypic, functional, and comparative studies with monoclonal antibodies," *Journal of Immunology*, vol. 133, no. 1, pp. 180–189, 1984.

[40] A. Almeida-Oliveira, M. Smith-Carvalho, L. C. Porto et al., "Age-related changes in natural killer cell receptors from childhood through old age," *Human Immunology*, vol. 72, no. 4, pp. 319–329, 2011.

[41] I. Gayoso, B. Sanchez-Correa, C. Campos et al., "Immunosenescence of human natural killer cells," *Journal of Innate Immunity*, vol. 3, no. 4, pp. 337–343, 2011.

[42] E. G. Addison, J. North, I. Bakhsh et al., "Ligation of CD8alpha on human natural killer cells prevents activation-induced apoptosis and enhances cytolytic activity," *Immunology*, vol. 116, no. 3, pp. 354–361, 2005.