

Role of Exercise-Induced Oxidative Stress in Sickle Cell Trait and Disease

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Abstract Sickle cell disease is a class of hemoglobinopathy in humans, which is the most common inherited disease in the world. Although complications of sickle cell disease start from polymerization of red blood cells during its deoxygenating phase, the oxidative stress resulting from the biological processes associated with this disease (ischaemic and hypoxic injuries, hemolysis and inflammation) has been shown to contribute to its pathophysiology. It is widely known that chronic exercise reduces oxidative stress in healthy people, mainly via improvement of antioxidant enzyme efficiency. In addition, recent studies in other diseases, as well as in sickle cell trait carriers and in a mouse model of sickle cell disease, have shown that regular physical activity could decrease oxidative stress. The purpose of this review is to summarize the role of oxidative stress in sickle cell disease and the effects of acute and chronic exercise on the pro-oxidant/antioxidant balance in sickle cell trait and sickle cell disease.

Key Points

The pathophysiological effects of sickle cell disease, such as hemolysis, endothelial dysfunction, inflammation and vaso-occlusion, are associated with altered levels of reactive oxygen species and antioxidants.

Acute and chronic exercise can alter the pro-oxidant/antioxidant balance in sickle cell trait and sickle cell disease, favoring a reduction in pathophysiological complications.

1 Introduction

Sickle cell disease (SCD) is a hemoglobinopathy resulting from a single mutation in the *HBB* [hemoglobin, beta] gene, inducing substitution of valine for glutamic acid at the sixth amino acid position. This mutation leads to production of abnormal (sickle) hemoglobin (HbS). Polymerization of deoxygenated HbS converts a normal red blood cell (RBC) into a rigid, irregular shaped, unstable cell. Sickling of RBCs results in deleterious pathophysiological effects, such as hemolysis, endothelial dysfunction, inflammation and vaso-occlusion. These independent manifestations are associated with altered levels of reactive oxygen species (ROS) and antioxidants. Endothelial dysfunction, due to impairment of nitric oxide (NO) bioavailability, is characterized by increased vascular adhesion, leading to vaso-occlusion and hypoxic episodes [1, 2]. These consequences can further generate superoxide

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and inhibit NO production while generating additional ROS [3].

The purpose of this review is to describe the role of exercise-induced oxidative stress in the pathogenesis of sickle cell trait (SCT) and SCD. The review first provides an overview of the role of oxidative stress in SCT and SCD, followed by a concise review of the current literature describing how both acute and chronic exercise affect generation of oxidative stress, antioxidants and NO in both conditions.

2 Oxidative Stress in Sickle Cell Disease: A Multifactorial Process

The cyclic nature of SCD pathology involves impairment of the pro-oxidant/antioxidant balance in favor of the former via increasing levels of ROS or decreasing antioxidant defenses [4, 5]. Several studies have reported elevated lipid peroxidation and protein oxidation levels (end-products of oxidative reactions as a marker of oxidative stress) in plasma, serum and erythrocytes in SCD patients [6–12].

Simultaneously, the antioxidant protective mechanisms are usually decreased in SCD patients [7, 13–17]. Those that provide enzymatic defense, including superoxide dismutase (SOD) [18, 19], glutathione peroxidase (GPX) [16], catalase [18] and heme oxygenase (HO)-1, and those that scavenge free radicals, such as glutathione (GSH), vitamin C and vitamin E, are most affected [8, 20].

2.1 Alteration of Antioxidant Pathways

One study reported that SCD patients had a 40 % reduction in carotenoids and a 30 % reduction in vitamin E in plasma [8]. In that study, the lower plasma concentrations of vitamins (A, E and C) in SCD patients were associated with an impaired blood total antioxidant status measured by free oxygen radical defense. The decrease in GSH content (20–50 %) in RBCs [8, 15, 20], platelets, neutrophils [20] and plasma [15] in these patients is also of major importance because this compound is an essential co-factor of GPX to reduce H₂O₂.

Studies have also shown a decrease in the activities of GPX [14, 16] and catalase [14, 21], two enzymes involved in the reduction of H₂O₂ to H₂O and oxygen. Similarly, several studies have reported a decrease in SOD activity in SCD patients [19] and murine models of SCD [18]. This may result in excessive ROS production, which could quickly consume or inactivate these enzymes. The decrease in antioxidant enzymatic activity could be explained by overconsumption of substrates [20] and co-factors [16] of these enzymes or their down-regulation due to an exaggerated stimulus, as has been shown during overtraining

syndrome in athletes [22]. The antioxidant enzyme activity impairment may thus, in turn, participate in the increase in oxidative stress markers usually observed in SCD.

However, other studies have shown that enzymatic activities of SOD in RBCs [8, 9, 14] and serum [21] were higher in SCD patients, likely resulting from stimulation of antioxidant defenses in response to the increased oxidative stress in SCD. Moreover, a study by Belini Junior et al. [23] recently showed that plasma antioxidant status, measured by Trolox equivalent antioxidant capacity, increased concomitantly with the increase in lipid peroxidation in SCD patients. Similarly, Goswami and Ray [21] reported a positive correlation between lipid peroxidation levels and the activity of SOD in serum. These increases in blood antioxidant defenses may result from ROS overgeneration in SCD.

2.2 Pathophysiology of Reactive Oxygen Species Generation

Figure 1 synthesizes the physiological processes that lead to oxidative stress increase in SCD.

2.2.1 Sickle Hemoglobin Auto-oxidation

In the presence of oxygen, heme auto-oxidizes, inducing methemoglobin and superoxide formation. Although both normal hemoglobin (HbA) and HbS have a tendency to autoxidize into these byproducts [13, 24], some studies have shown that HbS can auto-oxidize 1.7 times faster than HbA [25, 26], while others have shown a comparable rate [27]. While HbA can limit the occurrence of this reaction and subsequent oxidative products, autoxidation of HbS produces high levels of superoxide anion and hydrogen peroxide via a reaction of dismutation. RBCs from SCD patients have a significantly higher RBC ROS content [28], and sickled cells ultimately generate about two-fold greater quantities of superoxide (O₂^{•-}), hydrogen peroxide (H₂O₂) and hydroxyl radical (OH[•]) than normal RBCs [20].

2.2.2 Hemolysis: Heme, Free Hemoglobin and Iron

Source of ROS in SCD can also stem from the hemolysis process via release of iron or iron compounds, such as free hemoglobin and heme, into plasma [20, 29]. Furthermore, haptoglobin and hemopexin, two important scavengers of cell-free hemoglobin and heme, respectively, are depleted in SCD patients [30, 31].

Because of its hydrophobicity characteristic, free heme can accumulate within the cell membrane of RBCs, leading to membrane oxidative damage [29] and decompartmentalization of iron [13]. When a reducing agent is present, decompartmentalized iron promotes lipid and protein

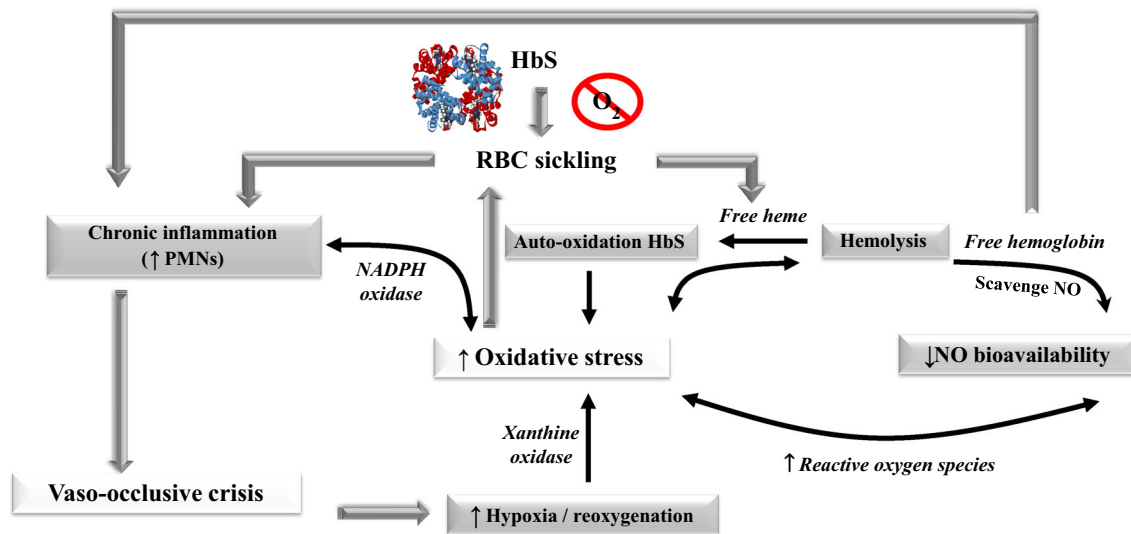


Fig. 1 Oxidative stress pathways in sickle cell disease. The hemolysis process and subsequent release of heme, free hemoglobin and iron; auto-oxidation of sickle hemoglobin (HbS); hypoxia/reoxygenation stimuli via the xanthine oxidase pathway; and vascular inflammation via production of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in polymorphonuclear neutrophils (PMNs) all increase oxidative stress in sickle cell anemia. Reactive oxygen species produced in excess can alter NO bioavailability,

increase inflammation and exacerbate red blood cell (RBC) sickling. Free hemoglobin can also interact with NO, reducing NO expression and enhancing the chronic inflammatory state. Consequently, oxidative stress plays a central and key role in alteration of these pathophysiological parameters, leading to a vicious cycle, which increases the risk of triggering painful vaso-occlusive crises in sickle cell disease. ↑ increase, ↓ decrease

oxidations via Haber–Weiss reactions, which amplify ROS production of hydroxyl radicals (OH[•]). Free heme can also initiate a cascade of oxidative reactions [32]. It has been shown in vivo that an excess of hydrogen peroxide can bind to the heme moiety, producing fluorescent degradation products, which cause erythrocyte oxidative stress [33]. Cell-free hemoglobin also has a large impact on the bioavailability of NO, resulting in endothelial dysfunction and vasoconstriction. Finally, although humans and transgenic mice have been shown to up-regulate HO-1 in response to heme-induced oxidative stress, SCD patients have insufficient activity of HO-1 to handle the burden of heme [29].

2.2.3 Fate of Nitric Oxide

NO is an important regulator of vascular tone, blood flow, vascular adhesion, platelet aggregation and RBC rheology [34, 35]. In SCD patients, generation of NO dramatically decreases and the amount available for use is limited as well. The bioavailability of NO can be reduced in several ways: (1) through its consumption by the free radical superoxide; (2) through the products of hemolysis (free heme and arginase); and (3) through ‘uncoupling’ of endothelial NO synthase (eNOS; Fig. 2).

The interaction of superoxide and NO can produce more damage than either can alone. In normal systems, superoxide can easily be dismutated by SOD; however,

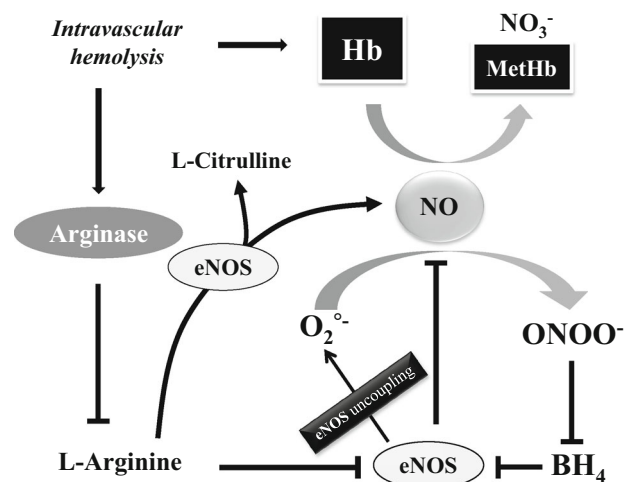


Fig. 2 Degradation of nitric oxide. NO is decreased in three ways: through reaction with superoxide (O₂^{•-}), through formation of peroxynitrite (ONOO⁻) and through its inhibition via the byproducts of hemolysis. BH₄ tetrahydrobiopterin, eNOS endothelial nitric oxide synthase, Hb hemoglobin, MetHb methemoglobin

overproduction of O₂^{•-}, which is common in SCD, can overwhelm the body’s antioxidant systems. NO and superoxide can react at diffusion-limited rates and can result in generation of peroxynitrite (ONOO⁻), a powerful and highly reactant oxidant. Generation of ONOO⁻ is favored over spontaneous superoxide dismutation and NO

autoxidation; the interaction is faster than both NO's reaction with hemoglobin and superoxide's reaction with SOD [13]. While RBCs from SCD patients have increased endothelial-like NOS activity, in comparison with those from control subjects, the large amount of NO that is formed is rapidly converted into ONOO⁻ [35]. ONOO⁻ can then form two other potent oxidants, OH[·] and nitrogen dioxide (NO₂) [13]. The reaction between NO and O₂^{·-} is two-fold in that it not only further decreases the concentration of NO but also produces more reactive free radicals.

As mentioned in Sect. 2.2.2, the hemolysis process increases the concentration of plasma hemoglobin, which allows for reaction of both deoxy- and oxy-HbS with NO, leading to a decrease in NO bioavailability. When NO binds to deoxygenated hemoglobin (Hb), a stable Fe²⁺Hb-NO complex forms, which can readily react in Fenton reactions [29]. The reaction between NO and oxygenated hemoglobin can form methemoglobin and nitrate. It has been reported that SCD patients have much higher levels of cell-free hemoglobin and methemoglobin, resulting in consumption and decreased bioavailability of NO [36–40]. It has been shown that, in contrast to the normal blood heme level of 0.2 μM, SCD patients have up to 20 μM of heme in their blood, which can increase several-fold during a crisis [38]. Reiter and Gladwin [36] reported that in SCD patients, those with plasma heme concentrations of 6 μM or greater had 80 % decreased NO-dependent vascular reactivity.

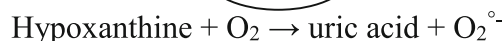
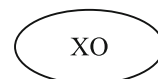
NO is further reduced through the consequence of eNOS 'uncoupling'. eNOS is an enzyme containing a reductase domain and an oxygenase domain, which produces NO. Normally, through the catalytic action of eNOS, tetrahydrobiopterin (BH₄) transfers an electron to the oxygenase domain, converting L-arginine into NO and L-citrulline. However, under certain conditions, eNOS 'uncoupling' can occur, producing superoxide rather than NO. Both of the main co-factors of this mechanism, L-arginine and BH₄, are reduced in SCD, thereby producing superoxide [39, 41]. In some SCD patients, L-arginine levels are significantly decreased [39], coinciding with an almost two-fold increase in arginase levels [37, 39, 42]. The use of L-arginine supplements in the diets of both mice and humans highlights the important role of L-arginine in NO production. Supplementation in SCD mice has been shown to reverse NO resistance [43], increase NO bioavailability and increase antioxidant activity [18]. These studies demonstrate a close relationship between decreased arginine, hemolysis and NO levels. Another important co-factor of eNOS, BH₄, is also affected by the repercussions of SCD, leading to superoxide generation. In sickle transgenic mice, it was demonstrated that a decrease in BH₄ was responsible for eNOS uncoupling [41]. ONOO⁻ led to production of O₂^{·-} in the vessels of control mice in contrast to eNOS-

deficient mice, while increasing aortic lipid peroxides in rats [44]. The combined effects of these studies demonstrate the vital role of BH₄ in production of NO.

2.2.4 Hypoxia/Reperfusion Injury: Xanthine Oxidase Pathway

The intracellular polymerization of HbS during deoxygenation is the primary pathogenic event in SCD. Polymerization transforms an apparently 'normal' RBC into a dense and inflexible sickled RBC [45, 46]. Hypoxia is a common repercussion of SCD and is due to adhesion and vaso-occlusion. Limitation or cessation of blood flow to tissues causes an ischaemic or hypoxic environment. During this state, limited oxygen is available to tissues and inadequate amounts of nutrients are available to support metabolic needs. ROS can be generated at various points: during hypoxia in both the mitochondria and in tissues, as well as during the reperfusion phase that follows. This phase can lead to reperfusion injury—damage caused to the vessel and tissue when oxygen is reintroduced to the tissues, leading to an increase in the concentrations of radical species [47].

Under hypoxic conditions, adenosine triphosphate (ATP) is reduced to adenosine diphosphate (ADP) and adenosine monophosphate (AMP). If the oxygen supply continues to decrease below certain levels, AMP is catabolized, leading to accumulation of hypoxanthine and xanthine in the tissue [47, 48]. The xanthine oxidase (XO) that is then produced can lead to deleterious effects due to the restitution of blood flow and therefore oxygen to the cells. During reoxygenation in these tissues, oxygen can react with the hypoxia-formed XO, resulting in damaging effects of oxidative stress. This reaction leads to conversion of hypoxanthine and xanthine by XO into superoxide [47–49].



A study by Osarogiagbon et al. [48] on reperfusion injury in sickle mice found a significantly higher proportion of XO during hypoxic conditions, in comparison with control or sickle mice exposed to ambient air. As supported by the equation above, this increase in hypoxia-generated XO can lead to production of O₂^{·-}. The increase in O₂^{·-} can lead to generation of free radicals as observed by increases in ethane excretion and OH[·], two markers of oxidative stress. To further verify that the increase in oxidative stress was indeed a result of XO, the addition of an XO inhibitor, allopurinol, decreased ethane excretion levels [48].

2.2.5 Chronic Pro-inflammatory State: Role of NADPH Oxidase

While $O_2^{\cdot-}$ is derived mainly from the XO pathway in hypoxic conditions, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase has also been shown to be an important source of $O_2^{\cdot-}$ in vascular tissue in normoxia [25]. ROS can be produced from binding of NADPH oxidase on endothelial cells in normoxia and also from activated polymorphonuclear neutrophils via an NADPH oxidase-mediated respiratory burst in inflammatory conditions.

3 Effects of Exercise

In healthy subjects, aerobic exercise can cause an increase of up to 15-fold in the rate of oxygen consumption throughout the body, and as much as 100-fold in the oxygen flux in active muscles [50]. As oxygen consumption and flow increase, so does the possibility of ROS production. As a consequence, acute bouts of exercise induce ROS production via several pathways, such as an increase in mitochondrial oxygen flux [50, 51], ischemia/reperfusion stress, an inflammatory response, NADPH oxidase activity and auto-oxidation of catecholamines [52]. The consensus of most human and animal studies is that acute aerobic exercise [53–55] and anaerobic exercise [55, 56] increase oxidative stress, although high-intensity aerobic exercise is likely the modality that generates the most ROS. In 1978, Dillard et al. [57] were the first to find that exercise at 75 % of maximal oxygen uptake (VO_{2max}) increased levels of lipid peroxidation, in comparison with resting subjects. It was then shown that exhaustive exercise increased liver and muscle free radical concentrations two- to three-fold [53]. Long-duration exercise also increases free radical concentrations in skeletal muscle and myocardium [58]. However, lack of or a lesser increase in oxidative stress in response to acute exercise can be due to a lower intensity [57, 59] and a shorter duration [58] of the exercise protocol, lower training status [60–62] and older age [63] of the subjects. More specifically, oxidative stress was shown to be negatively associated with the fitness level in various populations [64, 65]. In response to acute physical activity, the body's antioxidant defense system may be temporarily reduced as it combats the increase in ROS. During exercise and immediately after the exercise bout, antioxidants may be reduced [66, 67], indicating an increase in ROS. Further into the recovery period, antioxidants may increase above basal levels [63, 68, 69], indicating a return to more normal levels of ROS.

Regarding the effect of chronic exercise training, it has been reported that trained healthy subjects had lower levels

of oxidative stress at rest [70]. Most studies have demonstrated a reduction in post-exercise oxidative stress after endurance exercise training [71, 72]. In a rat model, exercise training was able to reduce oxidative stress in various tissues, such as the brain, liver and muscle, indicating that the effects of exercise involve a system-wide response [73]. A possible mechanism for the reduction in oxidative stress is up-regulation of NO and enzymatic antioxidants. Over an extended period of exercise training, cells may activate *de novo* synthesis of antioxidant enzymes to manage oxidative stress. As one of the initial antioxidants to respond to oxidative stress, SOD has often been reported to increase in response to exercise training [74–76]. Catalase activity in skeletal muscle in response to exercise training has inconsistent results. Different studies have shown an increase [75, 76], a decrease [74, 77] or no change [78]. The activity of GPX demonstrates a muscle fiber-specific pattern, with type 2a being the most responsive to training [79]. With regard to non-enzymatic antioxidants, α -tocopherol has been shown in animal models to decrease in skeletal muscle (specifically, fast-twitch muscle [73]), the liver and the heart [80]. Non-enzymatic antioxidants are also altered in other tissues. In rats, ascorbic acid was shown to increase in the brain but decreased in fast- and slow-twitch muscle, while ubiquinone decreased in slow-twitch muscle [73].

Exercise is a stimulus that also induces marked metabolic changes, such as acidosis, dehydration, hyperthermia, intermittent tissue hypoxia and organ hypoxia; all of these parameters are involved in the vicious cycle of SCD. Exercise-induced hyperthermia and dehydration have been shown to increase oxidative stress and impair antioxidant production [81, 82]. Metabolic acidosis, as a result of hypoxia or hypercapnia, has also been shown to increase oxidative stress [83]. Independent of acidosis, hypoxia exposure was also shown to increase circulating oxidative stress in healthy subjects [84]. More specifically, hypoxia exposure generates ROS by down-regulating mitochondrial complex III, leading to an increase in electrons escaping from the electron transport chain, activation of XO and NADPH oxidase pathways, and eNOS inhibition, leading to eNOS 'uncoupling'.

In conclusion, the temporary physiological disturbances induced by acute exercise could be problematic in SCD patients because of the increased rates of HbS polymerization and RBC sickling, which may potentially precipitate vaso-occlusive crises (VOCs). Conversely, regular exercise has been shown to be beneficial because it improves vascular function and antioxidant enzymatic activities in healthy subjects [85] and patients with chronic diseases [86]. Nevertheless, the effects of chronic physical activity in SCD patients are yet to be examined [87].

3.1 Exercise in Sickle Cell Trait

SCT is the heterozygous form of SCD and is characterized by the presence of both HbA (>50 %) and HbS. SCT is usually considered to be benign and asymptomatic, as numerous studies have reported normal growth and development, and normal morbidity and mortality [88]. However, several authors have suggested that it should be reclassified as a disease state [89, 90]. In a rebuttal position statement [89], and more recently in an editorial [91], Connes et al. stated that metabolic or environmental changes, such as hypoxia, acidosis or dehydration, could be responsible for the change from a silent condition into one resembling SCD, with VOCs resulting from an increase in RBCs with reduced deformability in the microcirculation. Furthermore, numerous studies have reported exercise-related sudden deaths of SCT carriers [88, 92–97], possibly due to RBC abnormalities associated with an increase in oxidative stress [14, 98].

Most carriers of SCT are able to perform exercise and sport as normal athletes would [99]. However, because of the high incidence of sudden death in army recruits and athletes, it is important to understand how an acute bout of exercise may complicate the disease.

3.1.1 Acute Exercise in Sickle Cell Trait

It is unclear whether oxidative stress and NO metabolism are impaired in SCT carriers. Although few studies have looked at changes in oxidative stress in this context, the evidence suggests that there is little difference between SCT carriers and control subjects in response to exercise or during recovery [100, 101]. Nonetheless, a slight trend towards increased susceptibility to oxidation in SCT erythrocytes during exercise has been observed [101]. In addition, Faës et al. [102] found that SCT carriers had an increase in advanced oxidation protein products (AOPPs) in response to exercise, while malondialdehyde (MDA) levels remained higher during recovery than in control subjects. However, no change in NO metabolism was observed between the subject groups or in response to exercise [100, 102]. In terms of antioxidant capacity, it was found that SOD and catalase activities were similar in both SCT carriers and control subjects in response to exercise and recovery [101, 102]. However, GPX and two NADPH-generating enzymes, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, remained less active in SCT carriers and did not respond to exercise as they did in the control subjects. In contrast, Faës et al. [102] found no difference between SCT carriers and control subjects in terms of GPX activity. The lack of induction of NADPH-generating enzymes due to exercise training in SCT carriers may explain why SCT carriers

have a slight susceptibility to oxidation. The difference in oxidative stress results could be due to the exercise intensities. In the studies by Tripette et al. [100] and Das et al. [101], subjects performed a submaximal exercise test, while in the study by Faës et al. [102], the individuals performed a maximal exercise test. As ROS production is known to be dependent on exercise intensity [50], maximal exercise may be required to observe oxidative stress and NO metabolism impairment as proposed for healthy subjects [103, 104] and trained subjects [105]. Therefore, it is essential to test SCT carriers during high-intensity exercise to determine the potential role of oxidative stress in sudden death in these subjects.

3.1.2 Chronic Exercise in Sickle Cell Trait

There have been few cross-sectional studies that looked at the effects of a training protocol on SCT carriers [106–108]. In these studies, trained SCT carriers (doing at least 8 h of sport training per week) and untrained SCT carriers were compared with trained and untrained control subjects. Various markers were evaluated in response to a maximal exercise test.

In a study by Vincent et al. [107], it was shown that exercise-trained SCT carriers have an altered oxidative potential, in comparison with control subjects, as measured by a different mitochondrial respiratory chain complex content. Remodeling of the muscle microvascular network is proportionally maintained between trained and untrained SCT carriers and control subjects, as similar improvements have been observed in capillary density and the number of capillaries around a single fiber [107].

Only one study has looked at the relationship between exercise training in SCT and oxidative stress. In response to a maximal incremental exercise test, SCT carriers exhibited higher levels of oxidative stress than healthy subjects. However, exercise training was able to reverse the increase in markers of oxidative stress seen in untrained SCT subjects. In addition, markers of antioxidants and NO were elevated in those SCT carriers who exercised versus those who did not [108]. This study adds to the conceptual idea of how exercise training could be beneficial to the SCT population, as oxidative stress can initiate the inflammatory response and mediate adhesion molecules [106].

3.2 Exercise in Sickle Cell Disease

It is uncertain whether physical activity is safe or effective in SCD patients [87]. The presence of anemia may induce a faster transition from aerobic to anaerobic metabolism during exercise, leading to polymerization and sickling of HbS [109]. Dehydration and temperature changes

occurring during exercise are other concerns that may contribute to RBC sickling [87]. Thus, because of the increased risk of VOCs [87] associated with unclear and confusing recommendations, physicians are hesitant to recommend physical activity in SCD patients. The studies mentioned below constitute a first step in the process of understanding the effects of such moderate exercise on oxidative stress markers and NO bioavailability.

3.2.1 Acute Exercise in Sickle Cell Disease

In one study by Barbeau et al. [110], SCD women performed three repeated bouts of moderate-intensity treadmill exercise (65–75 % of peak heart rate) on three consecutive days. It was found that these SCD patients had higher levels of the vasoactive mediators endothelin-1 and NO at baseline. At the end of day 3, NO metabolism was greater in SCD patients, with no change in NO metabolism or endothelin-1 levels throughout the protocol in control subjects. These results suggest that short exercise training is well tolerated by SCD patients and may be necessary to induce NO and improve vascular function, as was shown in SCT [106].

A study by Faës et al. [111] investigated the modulation of oxidative stress in response to a mild–moderate cycling test lasting 20 min. Mild changes in oxidative stress markers, antioxidant enzymes and NO bioavailability were found in SCD patients compared with control subjects in response to the exercise test. Only lipid peroxidation (MDA levels) increased in both groups after this exercise, while no statistical differences were observed between control and SCD patients. However, lower concentrations of NO were measured in SCD subjects at rest and in response to the exercise test, in comparison with the control group. This occurred concomitantly with an increase in nitrotyrosine, a measure of NO-consuming peroxynitrite. Therefore, with regard to these results and the lack of complications during and throughout the 3 days following this test, SCD patients could practice this kind of exercise without risk hazard. The intensity (under the first anaerobic threshold) and the short duration (i.e. 20 min) were chosen because these exercise modalities were previously shown to be safe and may even improve hemorheological parameters [112, 113]. Intensities higher than the anaerobic threshold may increase RBC sickling and thus the risk of vaso-occlusion via augmented acidosis [109].

3.2.2 Chronic Exercise in Murine Models of Sickle Cell Disease

Little information is available on the role of chronic exercise in patients with SCD. As a means to investigate the precise mechanisms within this disease without risking

the health of patients, researchers are turning to transgenic SCD mice.

Two recent studies observed the effects of voluntary exercise training (for 8 weeks) in SCD (SAD) mice on the response to acute hypoxia/reoxygenation (H/R) stress [114, 115]. The study found higher levels of NO metabolites, NOS3 [nitric oxide synthase 3 (endothelial cell)] messenger RNA and phosphorylated NOS3 expression in the lungs, and lower oxidative stress marker (MDA) levels in the hearts of exercise-trained SAD mice in response to H/R stress. Furthermore, exercise training increased cardiac antioxidant enzyme (GPX) activity at baseline and decreased plasma lactate dehydrogenase (LDH) concentrations—a marker of hemolysis—after H/R stimulus in physically active SAD mice. These results demonstrate that a chronic exercise-induced reduction in oxidative stress may decrease the risk of triggering VOCs in these mice. Nevertheless, the effects of chronic physical activity in SCD patients have not yet been examined, and future studies should test the effects of a moderate-intensity training program on oxidative stress and other biological markers of vascular complications in SCD patients.

4 Conclusion

The impaired redox status in SCD constitutes both a cause and a consequence of VOCs in this pathology [4, 5]. Simultaneously, chronic physical activity is known to improve resistance to an excess of ROS by increasing antioxidant defenses [64, 65, 116, 117].

SCT carriers experience impaired plasma oxidative stress levels in response to an acute exercise bout, which could be reversed by chronic exercise training. Conversely, sickle cell patients exhibit higher oxidative stress and lower NO bioavailability at rest than healthy subjects, and only slight changes have been observed after mild to moderate exercise, suggesting its safety. Nonetheless, except for two case studies [118, 119] that reported improvements in quality of life, and a recent study [120] demonstrating improved muscular function in SCD patients after aquatic and land physical therapy, there is no information in the literature regarding the biological and vascular effects of training as a therapy in these patients. However, promising studies have shown that voluntary exercise in a mouse model of SCD could improve oxidative stress balance and NO bioavailability. These beneficial effects in a transgenic murine model, associated with those found in SCT carriers in response to exercise training, could translate well to SCD patients. Similarly to what has been observed in other metabolic and cardiovascular diseases, such an exercise training program may decrease oxidative stress and its associated pathophysiological mechanisms in SCD. The

current literature suggests that submaximal exercise does not induce medical complications in SCD patients and does not worsen oxidative stress more than it does in healthy subjects. Further clinical trials will be needed to confirm these preliminary findings and delineate the exercise intensity, exercise duration and environmental conditions that can be used safely in this population.

Thus, it is important to carry out further studies testing the kind of exercise that sickle cell patients could sustain and repeat chronically without risks to their health. In addition, it would be of benefit to examine adhesion and inflammation associated with oxidative stress in the vicious cycle of SCD and the occurrence of VOCs and other complications.

Compliance with Ethical Standards

Conflicts of interest Erica Chirico, Camille Faës, Philippe Connes, Emmanuelle Canet-Soulas, Cyril Martin and Vincent Pialoux declare that they have no conflicts of interest that are relevant to the content of this review.

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