Endurance training improves oxygen uptake/demand mismatch, metabolic flexibility and recovery in patients with sickle cell disease

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Abstract

Patients with sickle cell disease (SCD) display lower slope coefficients of the oxygen uptake ($\dot{V}O_2$) *versus* work rate (W) relationship (delineating an O_2 uptake/demand mismatch) and a poor metabolic flexibility. Because endurance training improves the microvascular network and increases the activity of oxidative enzymes, including one involved in lipid oxidation, endurance training might improve the slope coefficient of the $\dot{V}O_2$ *versus* W curve and the metabolic flexibility of SCD patients. Endurance training may also contribute to improve patients' post-exercise cardiopulmonary and metabolic recovery. Fifteen patients with SCD performed a submaximal incremental test on a cycle ergometer before (SIT1) and after (SIT2) 8 weeks of endurance training. Minute ventilation ($\dot{V}E$), ventilation rate, heart rate, $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio, carbohydrate/lipid utilization and partitioning (including %Lipidox) and blood lactate concentration were measured during and after SIT1 and SIT2. At baseline, the slope coefficient of the $\dot{V}O_2$ *versus* W curve positively correlated with total hemoglobin, mean corpuscular hemoglobin and percentage of HbF. After training, the slope coefficient of the $\dot{V}O_2$ *versus* W curve was significantly higher and the increase in blood lactate concentration was delayed. If patients' energy metabolism apparently relied largely on carbohydrate sources during SIT1, %Lipidox tended to increase at low exercise intensities during SIT2, supporting a training-induced improvement of metabolic flexibility in patients with SCD. Post-exercise recovery of ventilation rate, $\dot{V}E/\dot{V}CO_2$, heart rate and blood lactate concentration was faster after training. We concluded that exercise training in patients with SCD: (i) ameliorated the oxygen uptake/demand mismatch, (ii) blunted the metabolic inflexibility, and (iii) improved post-exercise cardiopulmonary and metabolic responses.

Introduction

Sickle cell disease (SCD) is the most common severe genetic disease and hemoglobinopathy in the world.[1] SCD is caused by a mutation of the gene that encodes for β-globin, leading to the synthesis of an abnormal hemoglobin S (HbS). When deoxygenated, HbS can polymerize, giving red blood cells a particular sickle shape. Sickle red blood cells are (i) fragile, leading to important hemolytic anemia on the one hand, and (ii) rigid and adherent to the endothelium, disturbing hemodynamics, favoring entrapment of the sickle red blood cells in the microcirculation, and potentially progressing

into vaso-occlusion on the other hand.

These consequences of SCD (hemolytic anemia, endothelial adherence, altered hemodynamics and vaso-occlusion) act in concert with a rarefaction of the microvascular network[2] and an apparent tissue shunt[3] to limit oxygen transport and delivery deep into the tissues. The active skeletal muscle, which uses oxygen for its energy metabolism, may suffer particularly from this impeded oxygen supply. Additionally, the muscle remodeling associated with SCD includes alterations of the activity of oxidative enzymes[2] indicative of impaired mitochondrial respiration. Therefore, if the oxygen supply to skeletal muscles is restricted, their capacity to consume the oxygen is also limited.[2] As a consequence, SCD patients display poor physical ability as well as particular/abnormal responses to physical activity.[4,5] A first particular response to exercise is the shape of the oxygen uptake ($\dot{V}O_2$) *versus* work rate (W) relationship. Previous studies have shown that the slope of this relationship is lower in young adult[6] and adult[4,5] patients with SCD than in control subjects. This lower slope does not reflect a higher efficiency of movement in patients with SCD. Rather, the lower slope coefficient results from the disturbed oxygen delivery and utilization as illustrated by the lower total hemoglobin and peripheral oxygen extraction displayed by patients with SCD,[7] delineating an oxygen uptake/demand mismatch.[3] A second particular response to exercise in patients with SCD is the early and rapid accumulation of lactate in the blood.[4,8] If this early blood lactate accumulation signifies involvement of the glycolytic pathway in the energy supply, it also suggests a downregulation in lipid utilization. Indeed, elevated lactate levels inhibit lipolysis[9] and the function of carnitine palmitoyl transferase 1, the transporter of free fatty acids into mitochondria.[10] Because metabolic flexibility reflects the ability to oxidize carbohydrate and lipid during exercise,[13] poor metabolic flexibility can be suspected in the context of SCD.

In the past decade, studies have investigated the potentially beneficial effects of moderate-intensity endurance-exercise training programs in patients with SCD.[4,12-14] At the muscular level, this type of training enlarged the microvascular network and improved the activity of key mitochondrial energy metabolism enzymes.[14,15] Thus, endurance training seems to augment muscle oxygen supply and utilization. Whether these tissue adaptations lead to, or at least coincide with, alterations observable at the integrative level, such as a higher slope coefficient of the $\dot{V}O_2$ *versus* W relationship, remains unknown. Furthermore, the well-documented blunting effect of endurance training on blood lactate concentrations ($[lactate]_b$) in healthy subjects[16,17] occurs in patients with SCD,[14,15] suggesting that the lactate-related inhibition of lipolysis and free fatty acid entry in mitochondria may also be partially blunted. In this context, it is interesting to note that endurance training in patients with SCD increased the activity of 3-hydoxylacyl-CoA dehydrogenase, a key enzyme involved in β-oxidation.[15] Together, these latter results suggest a potential for increased lipid oxidation after endurance training in SCD, consequently improving metabolic flexibility.

Endurance training improves post-exercise physiological adaptations by accelerating the return to basal values in healthy subjects. These faster returns to baseline values are observable in cardiorespiratory parameters such as heart rate (HR), minute ventilation ($\dot{V}E$) and $\dot{V}O_2$, as well as in metabolic responses, including $[lactate]_b$.[16,18] For the cardiorespiratory parameters, these adaptations are particularly observable in the first part of the recovery.[18,19] However, whether similar post-training observations are present in patients with SCD has yet to be investigated.

The aim of the present study was, therefore, to test the hypotheses that in patients with SCD, endurance training would improve: (i) the oxygen uptake/demand matching, increasing the slope coefficient of the $\dot{V}O_2$ *versus* W curve, (ii) metabolic flexibility, and (iii) post-exercise cardiopulmonary responses.

Methods

Study population

Fifteen adult patients with homozygous SCD (HbSS or HbS/β^0-thalassemia genotypes; 7 women [2 taking hydroxyurea] and 8 men [7 taking hydroxyurea]) without severe chronic complications (see *Online Supplement*) participated in this study which took place in the Referral Center for Major Sickle Cell Syndrome in Créteil, France. They received no transfusions or supplemental oxygen during the whole duration of the study, nor were they hospitalized for vaso-occlusive crises. The study was approved by the ethics committee (Comité de Protection des Personnes Sud-Est 1 2014-14; EudraCT ID RCB 2014-A00334-43), conducted in accordance with the Declaration of Helsinki, and registered at *www.clinicaltrials.gov* (#NCT02571088). Volunteers were informed of the purposes, procedures, and possible associated risks and/or discomfort related to the protocol before they gave written informed consent. Part of the results have been previously published for other purposes.[8,14,15] When necessary, they are repeated here for the convenience of readers.

Study design

Patients were subjected to blood sampling (for complete blood count, HbS and HbF proportions, β-thalassemia, lactate dehydrogenase and total bilirubin) and performed the same submaximal incremental exercise test on a cycle ergometer before (SIT1) and after (SIT2) an 8-week endurance training program.

Submaximal incremental exercise test

The exercise test was performed on an electronic cycle ergometer (Ketler, Ense-Parsit, Germany). Exercise started at 20 W for women or 30 W for men and increased

stepwise every 2 min by 10 or 15 W for women or men, respectively. Gas exchange measurements (including ventilation rate [VR, cycle·min^{-1}], $\dot{V}E$ [L·min^{-1}], $\dot{V}O_2$ [L·min^{-1} or mL·min^{-1}·kg^{-1}] and carbon dioxide production [$\dot{V}CO_2$, L·min^{-1}]) and HR (beats·min^{-1}) were recorded continuously. Every minute, whole $[lactate]_b$ (mmol·L^{-1}) was assessed via a blood drop taken from the earlobe and analyzed extemporaneously (Lactate Scout, EKF diagnostics, Cardiff, UK). Exercise terminated as soon as a $[lactate]_b$ ≥4 mmol·L^{-1} was recorded.[4,14] The test was followed by 2 min of active recovery at 20 or 30 W for women or men respectively, and thereafter by at least 6 min of passive recovery. The recovery and observation period ended when experimenters observed both a clear decrease in $[lactate]_b$ and a $[lactate]_b$ value below 4 mmol·L^{-1}. This session was used to determine (*vide infra*) (i) indices of physical fitness, (ii) the $\dot{V}O_2$ *versus* W relationship, (iii) energy substrate oxidation and partitioning, (iv) cardiopulmonary data during recovery and (v) the initial target exercise intensity for the training sessions.

Endurance exercise training protocol

Patients completed a moderate-intensity endurance-exercise training period, composed of 24 exercise sessions (3 sessions a week for 8 weeks) on a cycle ergometer. Each training session started with an initial 5-min warm-up (at 70% of the target work rate), continued with a 30-min constant-load endurance exercise at the target exercise intensity, followed by a 5-min cool-down (at 70% of the target work rate), and ended with light stretching. During the training sessions, several parameters were recorded: HR, blood pressure, peripheral oxygen saturation and $[lactate]_b$. Patients were encouraged to drink water regularly for proper hydration. The exercise workload was selected with the goal of reaching a $[lactate]_b$ of ~2.5 mmol·L^{-1}. Depending on the $[lactate]_b$ obtained during each training session, exercise work rate for the subsequent training session was adjusted according to the strategy previously proposed.[4] A physician was present to observe the patients during each training session.

Blood lactate curve analysis

The blood lactate *versus* work rate curves obtained during SIT1 and SIT2 were used to identify (i) the first lactate threshold (LT1) defined as the first inflection point on the curve and (ii) the achievement of the 4 mmol·L^{-1} $[lactate]_b$. Work rate at LT1 was used as the initial target exercise intensity for the training sessions (expecting a $[lactate]_b$ of ~2.5 mmol·L^{-1})[4] while work rate at 4 mmol·L^{-1} of $[lactate]_b$ was used as a criterion for exercise termination.

Cardiopulmonary and gas exchange measurements and analyses

During SIT1 and SIT2, cardiopulmonary parameters (HR, VR, $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$) were measured continuously by an ErgoCard device (Medisoft, Sorinnes, Belgium). $\dot{V}O_2$ at LT1 ($\dot{V}O_{2@LT1}$) and at 4 mmol·L^{-1} blood lactate concentration ($\dot{V}O_{2@4mM}$) were used as physical fitness criteria. $\dot{V}O_2$ and $\dot{V}CO_2$ obtained at steady state (mean value of the last 20 seconds of steps of SIT, see *Online Supplement*) were considered for determination of the slope coefficient (a) of the linear $\dot{V}O_2$ *versus* work rate relationship and of the respiratory exchange ratio (RER) according to Eq. 1 and Eq. 2, respectively:

$$\dot{V}O_2 = a \cdot \text{work rate} + b \quad \text{(Eq. 1)}$$

$$RER = \dot{V}CO_2/\dot{V}O_2 \quad \text{(Eq. 2)}$$

Table 1. Some characteristics of patients (N=15).

Demography and anthropometry	SIT1	SIT2	*P*
Age in years	34.7±11.1	NA	-
Height, cm	172.1±10.5	NA	-
Body weight, kg	65.2±11.8	NM	-
Body mass index, kg·m^{-2}	22.8±2.9	NM	-
Hematology			
Hemoglobin, g·dL^{-1}	9.2±1.4	9.2±1.5	0.846
Hematocrit, %	27.3±4.2	27.1±4.5	0.741
HbS, %	79.5±8.7	80.8±8.6	0.044
HbF, %	11.1±8.7	10.7±8.1	0.612
MCV, fL	94.1±16.1	93.7±15.0	0.627
MCH, pg	31.7±5.9	31.7±5.4	0.882
MCHC, g·dL^{-1}	33.6±1.2	33.9±1.3	0.344
LDH, UI·L^{-1}	398±148	373±143	0.153
Reticulocytes, %	6.4±2.6	6.3±3.1	0.899
Total bilirubin, μmol·L^{-1}	38.1±28.1	36.6±22.8	0.783
Physical fitness parameters			
$W_{@LT1}$, W	38.5±10.8	54.8±14.5	<0.001
$\dot{V}O_{2@LT1}$, mL·min^{-1}·kg^{-1}	10.4±2.6	13.6±3.3	<0.001
$W_{@4mM}$, W	70.4±16.2	77.6±15.1	0.006
$\dot{V}O_{2@4mM}$, mL·min^{-1}·kg^{-1}	14.9±3.5	17.1±3.0	0.029
$th\dot{V}O_{2peak}$, mL·min^{-1}·kg^{-1}	41.7±6.4	NA	NA
$\%th\dot{V}O_{2peak@4mM}$, %	36.1±8.2	41.6±7.9	0.015
N</N> 52% $th\dot{V}O_{2peak@4mM}$	15/0	13/2	NA

Values are mean ± standard error. SIT1 and SIT2: submaximal incremental exercise tests 1 and 2, respectively; *P*: probability; NA: not applicable; NM: not measured; HbS: hemoglobin S; HbF: hemoglobin F; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; LDH: lactate dehydrogenase; $W_{@LT1}$ and $\dot{V}O_{2@LT1}$: work rate and oxygen uptake at the first lactate threshold; $W@_{4mM}$ and $\dot{V}O_{2@4mM}$: work rate and $\dot{V}O_2$ at the 4 mM blood lactate concentration i.e., at exercise cessation; $th\dot{V}O_{2peak}$: theoretical peak oxygen uptake; $\%th\dot{V}O_{2peak@4mM}$: percentage of $th\dot{V}O_{2peak}$ at 4 mM of blood lactate concentration; N: number of patients.

Carbohydrate oxidation (CHOox) and lipid oxidation (Lipidox) rates were assessed using, respectively, Eq. 3 and Eq. 4 proposed by Frayn.[20]

$CHOox\ (g{\cdot}min^{-1}) = 4.55 \cdot \dot{V}CO_2 - 3.21 \cdot \dot{V}O_2 - 2.87 \cdot 0.01$ (Eq. 3)

$Lipidox\ (g{\cdot}min^{-1}) = 1.67 \cdot \dot{V}O_2 - 1.67 \cdot \dot{V}CO_2 - 1.92 \cdot 0.01$ (Eq. 4)

Substrate partitioning via non-protein respiratory quotient (NPRQ) was calculated using equations proposed by Zarins *et al.*[21]

$NPRQ\ (\%) = [\dot{V}CO_2 - (0.01 \cdot 4.89)]/[\dot{V}O_2 - (0.01 \cdot 6.04)]$ (Eq. 5)

$\%CHOox\ (\%) = [(NPRQ - 0.707)/0.293] \cdot 100$ (Eq. 6)

$\%Lipidox\ (\%) = (100 - \%CHOox)$ (Eq. 7)

The last RER value taken into account for CHOox, Lipidox, %CHOox and %Lipidox analyses was the first value ≥1.0 but lower than 1.05 on SIT2.[11] For RER values ≥1.0, Lipidox and %Lipidox were considered to be null.

Theoretical peak oxygen uptake and related parameters

Theoretical peak oxygen uptake ($th\dot{V}O_{2peak}$) was calculated according to the equation proposed by Myers *et al.*[22] taking into account age, weight and gender as follows:

$th\dot{V}O_{2peak} = 79.9 - (0.39 \cdot age) - (13.7 \cdot gender) - (0.127 \cdot weight)$ (Eq. 8)

In this equation, $th\dot{V}O_{2peak}$ is expressed in $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ and weight in lbs. For gender, male = 0 and female = 1. $\dot{V}O_{2@4mM}$ was also expressed as percentage of $th\dot{V}O_{2peak}$ ($\%th\dot{V}O_{2peak@4mM}$). In the present study, the number of patients with values below and above 52% of $th\dot{V}O_{2peak}$ was considered. This cut-off percentage corresponds to 80% of the theoretical value of $\dot{V}O_{2@4mM}$ which is reached at 65% of $th\dot{V}O_{2peak}$ in healthy (active but untrained) populations.[17,23]

Recovery data analysis

Different variables (VR, $\dot{V}E$, $\dot{V}O_2$, HR and $[lactate]_b$) were measured and recorded at T0' and T2' of active recovery and at T0', T2', T4' and T6' of passive recovery. Differences between T0' and T2' of active recovery (Δ0-2) and between T0' and T6' of passive recovery (Δ0-6) were considered.

Statistical analysis

Statistical analyses were performed with Statistica (version 80.0, Statsoft, Tulsa, OK, USA). Values are presented as mean ± standard deviation. Normality of data distribution was tested and confirmed by the Shapiro-Wilk test. Differences between pre- and post-training data were investigated using a *t* test, dependent samples. Relationships between two different variables were studied by means of linear regressions (confirmed by Pearson tests). The level of statistical significance was set at α=0.05.

Results

Patients' characteristics

Some patients' baseline and post-training characteristics are reported in Table 1. Hemoglobin concentration and indirect markers of hemolysis (lactate dehydrogenase, reticulocytes, total bilirubin) were similar before and after training.

Submaximal incremental exercise

Step count was 4.8±1.0 and 5.6±1.2 for SIT1 and SIT2, respectively (*P*=0.004). The corresponding exercise duration was 9.5±2.0 and 10.9±2.1 min, respectively (*P*=0.003). Table 1 also reports pre- and post-training (SIT1 and SIT2, respectively) data of physical fitness parameters. More specifically, the work rates and $\dot{V}O_2$ at LT1 and at exercise completion ($W_{@LT1}$, $\dot{V}O_{2@LT1}$, $W_{@4mM}$ and $\dot{V}O_{2@4mM}$, respectively) as well as $\%th\dot{V}O_{2peak@4mM}$ were all significantly improved by endurance training.

Applying Eq. 1 to the individual experimental data, mean ± standard deviation r^2 value of the $\dot{V}O_2$ *versus* W correlation was 0.9492±0.0598. The slope coefficient of the curve was significantly higher after training (*P*=0.008) (Figure 1). Before training, the slope coefficient of the curve was positively correlated with the hemoglobin concentration, the mean corpuscular hemoglobin (MCH) content and the percentage of fetal hemoglobin (%HbF), and negatively

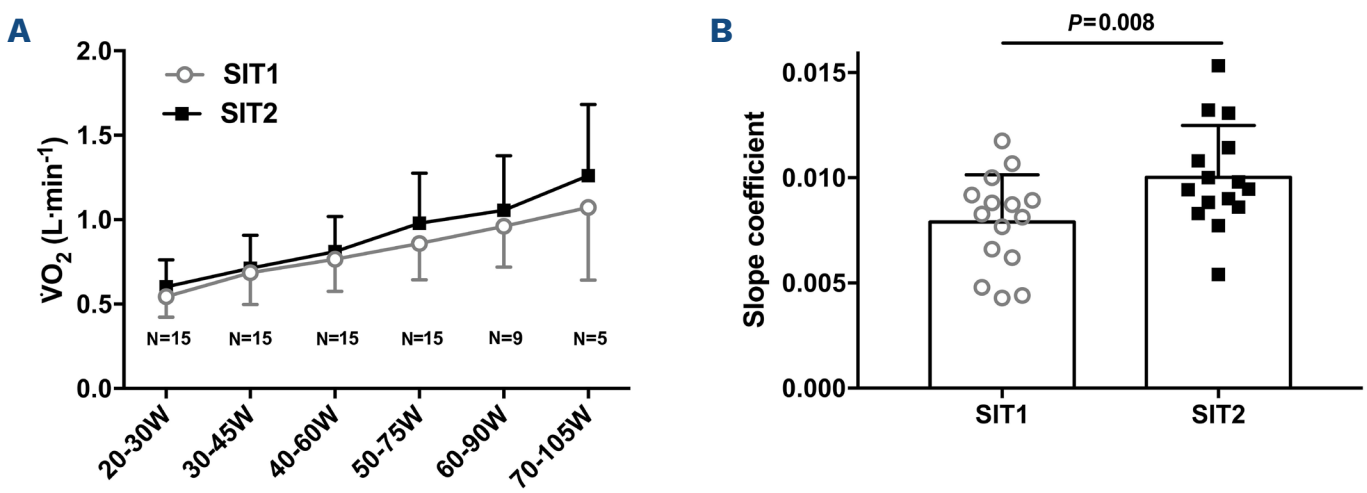

Figure 1. Oxygen uptake and work rate relationships. (A) Mean ± standard deviation of oxygen uptake *versus* work rate curves before and after training. (B) Slope coefficients of the oxygen uptake *versus* work rate relationship before and after training. Open dots and black squares are mean or individual values before and after training, respectively. $\dot{V}O_2$: oxygen uptake; SIT1: submaximal incremental test before training; SIT2: submaximal incremental test after training.

correlated with the percentage of hemoglobin S (%HbS) (Figure 2). After training, the slope coefficient was correlated with hemoglobin concentration (Figure 2).

Table 2 reports values of $[lactate]_b$, gas exchange measurements and substrate utilization and partitioning during SIT1 and SIT2. At rest and at 20/30 W, no training-induced differences were observed for $[lactate]_b$. At 30/45 W and 40/60 W, $[lactate]_b$ was significantly lower after training (P=0.005 and P=0.006, respectively) (Figure 3). At rest and all exercise intensities (20/30 W, 30/45 W, and 40/60 W), RER was significantly lower after training (P=0.03, P=0.004, P=0.004 and P=0.001, respectively). Lipidox increased at 30/45 W, while %Lipidox increased and thus %CHOox decreased at 30/45 W and 40/60 W.

Subsequent recovery

During active and passive recovery (Table 3), no significant changes were observed for $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$. During passive recovery (Δ0-6), the VR drop was greater after training (P=0.05).

HR decreased significantly more rapidly during active recovery after training, as shown by: (i) a lower mean HR after 2 min of active recovery (P=0.02), while HR at exercise completion was similar, and (ii) a greater post-training Δ0-2 of HR (P=0.02).

After training, $[lactate]_b$ decreased more rapidly during passive recovery (Table 3), as $[lactate]_b$ values at the 4th and 6th minutes of passive recovery were lower after training (P=0.05 and P=0.01, respectively).

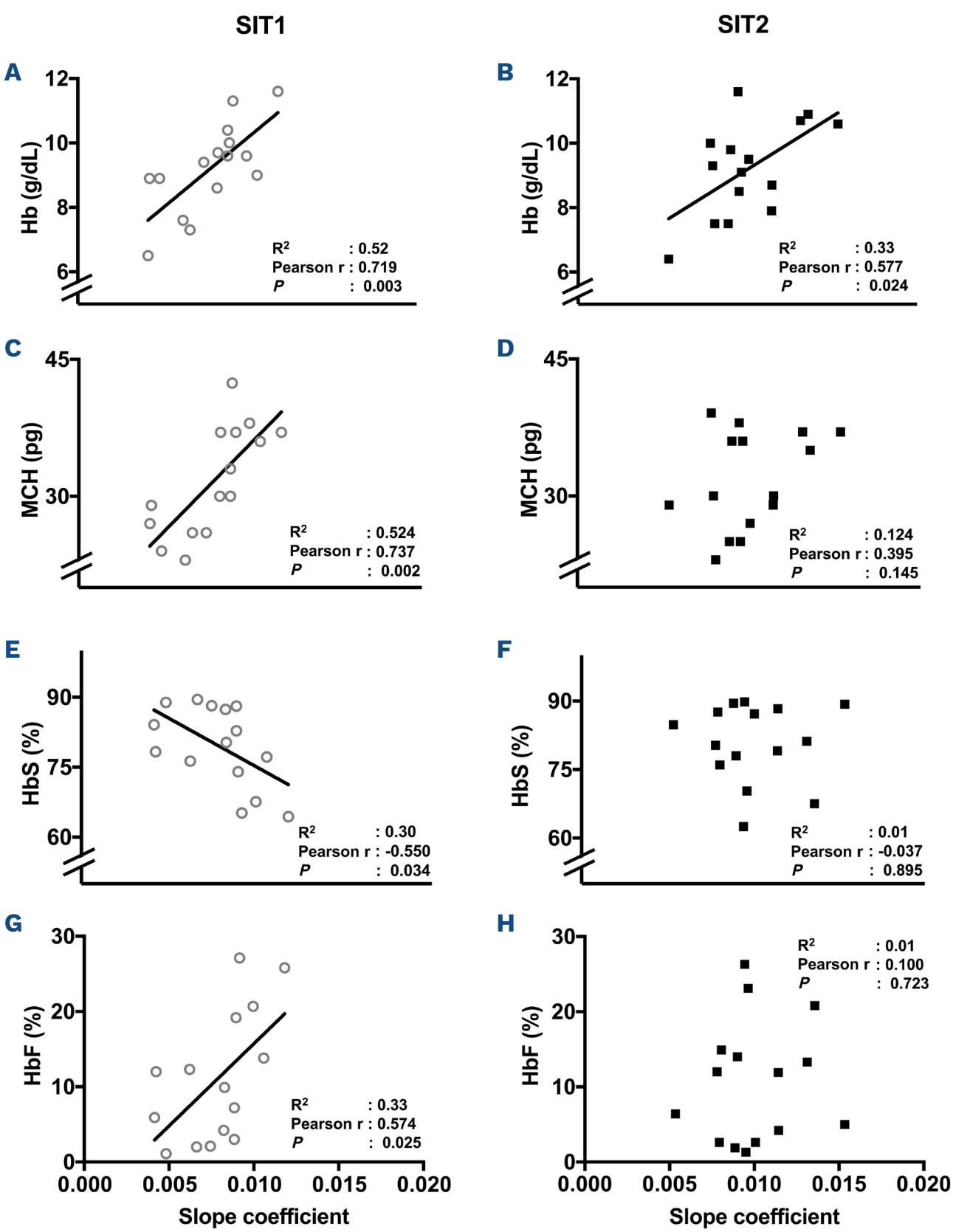

Figure 2. Correlations between the slope coefficient of the oxygen uptake *versus* work rate curves and total hemoglobin(A, B), mean corpuscular hemoglobin (C, D), and percentages of hemoglobin S (E, F) and F (G, H) before and after training. SIT1: submaximal incremental test before training; SIT2: submaximal incremental test after training; Hb: hemoglobin; MCH: mean corpuscular hemoglobin; HbS: hemoglobin S; HbF: hemoglobin F.

Discussion

The main findings of the present study were that 8 weeks of endurance training in patients with SCD: (i) increased the slope coefficient of the $\dot{V}O_2$ *versus* W relationship, (ii) blunted the metabolic inflexibility and (iii) improved post-exercise recovery of some cardiopulmonary and metabolic parameters.

Effects of endurance training on oxygen uptake/demand mismatch and blood lactate accumulation

The slope coefficient of the $\dot{V}O_2$ *versus* W relationship has been reported to be lower in young adult and adult patients with SCD than in control subjects.[4-6] This lower slope coefficient suggests an oxygen uptake/demand mismatch resulting from lower muscle oxygen supply due to anemia, microvasculature rarefaction and smaller capillary/fiber surface of exchange, and/or lower ability of muscle to consume oxygen as testified by the lower activity of oxidative enzymes in SCD patients.[2] In accordance with this inferred oxygen uptake/demand mismatch, blood lactate levels increased early (for very low exercise intensity) during incremental exercise in patients with SCD.[4] This oxygen uptake/demand mismatch at the whole-body level is reminiscent of the oxygen supply/demand mismatch at the cerebral and peripheral (hand and forearm) levels due to lower oxygen

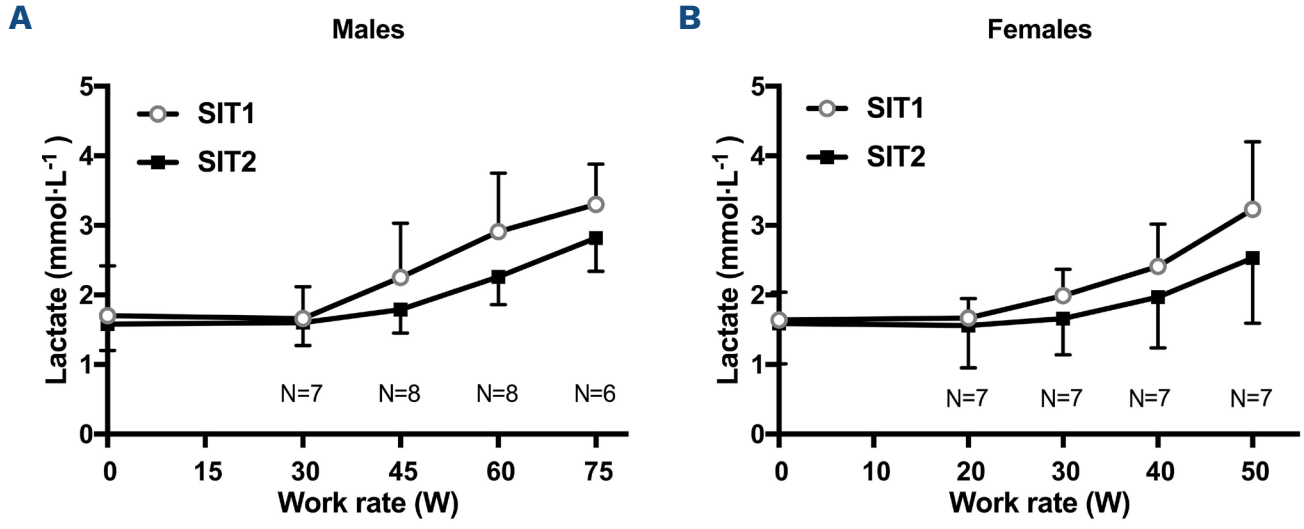

Figure 3. Blood lactate concentrations as a function of power output in males (A) and females (B) during submaximal incremental tests before and after endurance training. SIT1: submaximal incremental test before training; SIT2: submaximal incremental test after training.

Table 2. Blood lactate concentrations, gas exchange and substrate utilization and partitioning at different power outputs of the submaximal incremental tests before (SIT1) and after (SIT2) 8 weeks of endurance training.

Power output		$[lactate]_b$ mmol·L^{-1}	$\dot{V}O_2$ L·min^{-1}	$\dot{V}CO_2$ L·min^{-1}	RER	CHOox g·min^{-1}	Lipidox g·min^{-1}	%CHOox %	%Lipidox %
Rest	SIT1 SIT2	N=15 1.7±0.6 1.6±0.5 NS	N=12 0.33±0.1 0.28±0.1 NS	N=12 0.37±0.1 0.26±0.1 NS	N=12 1.12±0.2 0.93±0.1 *P*=0.03	N=12 0.3±0.2 0.3±0.1 NS	N=12 0.0±0.0 0.0±0.0 NS	N=12 99.7±26.5 95.9±31.6 NS	N=12 0.3±26.5 4.1±31.6 NS
20/30 W	SIT1 SIT2	N=14 1.7±0.5 1.6±0.5 NS	N=14 0.50±0.1 0.62±0.1 NS	N=14 0.55±0.1 0.56±0.1 NS	N=14 1.10±0.1 0.90±0.1 *P*=0.003	N=14 0.6±0.2 0.5±0.2 NS	N=14 0.0±0.1 0.1±0.1 NS	N=14 88.5±24.5 74.5±30.3 NS	N=14 11.5±24.5 25.5±30.3 NS
30/45 W	SIT1 SIT2	N=15 2.1±0.6 1.7±0.4 *P*=0.006	N=11 0.74±0.2 0.78±0.1 NS	N=11 0.72±0.2 0.70±0.1 NS	N=11 0.97±0.0 0.90±0.1 *P*=0.04	N=11 0.9±0.3 0.7±0.3 NS	N=11 0.0±0.0 0.1±0.1 *P*=0.04	N=11 96.4±11.2 72.2±30.1 *P*=0.04	N=11 3.6±11.2 27.8±30.1 *P*=0.04
40/60 W	SIT1 SIT2	N=15 2.7±0.8 2.1±0.6 *P*=0.005	N=7 0.80±0.2 0.90±0.2 NS	N=7 0.83±0.2 0.81±0.2 NS	N=7 1.04±0.2 0.90±0.1 *P*=0.01	N=7 0.9±0.2 0.8±0.2 NS	N=7 0.0±0.1 0.1±0.1 NS	N=7 93.7±14.0 69.4±18.2 *P*=0.02	N=7 6.3±14.0 30.6±18.2 *P*=0.02

Values are mean ± standard error. SIT1 and SIT2: submaximal incremental exercise tests 1 and 2, respectively; $\dot{V}O_2$: oxygen uptake; $\dot{V}CO_2$: CO_2 production; RER: respiratory exchange ratio; CHOox: carbohydrate oxidation; Lipidox: lipid oxidation; %CHOox and %Lipidox: substrate partitioning. N: number of patients; *P*: probability; NS: not significant.

extraction and testified by arterialization of venous blood in patients with SCD.[3,24] Interestingly, in the present study, the baseline slope coefficients were positively correlated with hemoglobin concentration, MCH and %HbF, as well as negatively correlated with %HbS (Figure 2A, C, E and G). These correlations suggest that, in our population, the baseline abnormal metabolic response to exercise (i.e., a low oxygen uptake for a given work rate) was associated with severity of anemia, and more generally with severity indices of the pathology.

Because endurance training enlarges the microvasculature, increases the capillary/fiber surface of exchange, and enhances the activity of oxidative enzymes (in other words, mitochondrial respiration) in patients with SCD,[8,15] we suspected improved oxygen supply and extraction/consumption after training. Consequently, exercise-associated energy metabolism is expected to rely more on oxygen-derived pathways after training. Therefore, we hypothesized that endurance training would reduce the oxygen uptake/demand mismatch by increasing the slope coefficient of the $\dot{V}O_2$ *versus* W curve in patients with SCD. The present results support this hypothesis (Figure 1). This assertion of a training-induced reduction of oxygen supply/demand mismatch via better oxygen supply (through an increased capillary network) and consumption (by increased mitochondrial respiration) also fits with the lower blood lactate accumulation (for a giver power output) after training (Table 2, Figure 3). This beneficial effect of endurance training on the slope resembles results obtained in trained athletes. Indeed, Lacour *et al*. showed that the most successful athletes displayed a higher slope coefficient of the $\dot{V}O_2$ *versus* W relationship associated with delayed blood lactate accumulation.[25] Of note, the observed increase in the slope coefficient after training was independent of any change in anemia since hemoglobin concentration was not altered by endurance training. Furthermore, after training, the correlation between the slope coefficients and hemoglobin was still present but those with MCH, %HbS and %HbF were not observed. These latter results suggest that if anemia remained a limiting factor for oxygen supply and consumption, the other indices of pathology seemed to be less determinant in the physiological responses associated with oxygen uptake during exercise after endurance training. The improved matching between oxygen uptake and demand is of paramount importance for patients with SCD. This adaptation promotes better physical ability allowing patients with SCD to perform more vigorous activities of everyday life (e.g., climbing stairs, carrying loads, walking faster).[3] The concomitant delay in blood lactate accumulation is equally significant because it should dampen

Table 3. Time courses of $\dot{V}E$, $\dot{V}E/\dot{V}CO_2$, VR, $\dot{V}O_2$, heart rate and blood lactate concentration during recovery following the submaximal incremental exercise tests 1 and 2 (SIT1 and SIT2).

		ACTIVE			PASSIVE				
		0	2	Δ 2-0	0	2	4	6	Δ 6-0
$\dot{V}E$ $L\cdot min^{-1}$	SIT1 SIT2	52.9±7.6 53.0±6.7 NS	35.5±5.8 34.3±5.7 NS	-17.4±7.9 -18.7±6.5 NS	31.2±4.6 32.0±5.9 NS	19.4±4.8 18.9±4.4 NS	17.1±4.4 16.8±4.5 NS	17.1±3.2 16.7±4.7 NS	-34.7±5.1 -36.5±4.8 NS
$\dot{V}E/\dot{V}CO_2$	SIT1 SIT2	41.8±6.3 39.4±6.1 NS	44.9±6.9 41.7±6.2 NS	3.1±3.1 2.3±2.4 *P*=0.001	43.7±6.0 42.3±6.6 NS	47.4±6.4 48.2±7.3 NS	49.8±4.2 50.0±5.7 NS	50.2±3.5 53.5±6.5 NS	1.0±18.3 5.1±20.3 NS
VR $cycle\cdot min^{-1}$	SIT1 SIT2	39.7±11.5 37.2±7.2 NS	35.4±11.4 30.0±6.8 *P*=0.04	- 4.4±6.6 -7.2±4.3 NS	30.6±8.6 28.8±6.0 NS	24.1±4.6 23.0±4.3 NS	21.5±4.6 21.4±3.9 NS	21.0±4.2 22.4±4.02 NS	-15.0±5.5 -14.1±4.7 NS
$\dot{V}O_2$ $L\cdot min^{-1}$	SIT1 SIT2	1.0±0.2 1.1±0.3 NS	0.7±0.2 0.7±0.2 NS	-0.3±0.2 -0.4±0.2 NS	0.6±0.2 0.7±0.2 NS	0.4±0.4 0.6±0.1 NS	0.3±0.1 0.3±0.1 NS	0.3±0.1 0.4±0.2 NS	-0.6±0.1 -0.8±0.2 *P*=0.02
HR $beats\cdot min^{-1}$	SIT1 SIT2	156.0±14.4 154.1±16.2 NS	131.6±14.7 124.5±10.1 *P*=0.02	-24.4±8.4 -29.6±7.5 *P*=0.02	126.5±14.4 121.6±9.8 NS	103.6±10 99.5±8.4 NS	97.5±10.4 95.7±9.3 NS	97.4±9.3 94.9± 10.4 NS	-28.0±12.1 -24.7±9.2 NS
$[lactate]_b$ $mmol\cdot L^{-1}$	SIT1 SIT2	4.8±0.6 5.0±0.6 NS	4.9±0.6 5.0±0.6 NS	0.0±0.4 -0.0±0.2 NS	4.8±0.7 5.0±0.6 NS	4.5±0.5 4.6±0.6 NS	4.1±0.6 4.2±0.8 *P*=0.05	4.1±0.4 3.9±0.5 *P*=0.01	-0.7±0.4 -1.3±0.5 *P*=0.01

Values are mean ± standard error. SIT1 and SIT2: submaximal incremental exercise tests 1 and 2, respectively; $\dot{V}E$: minute ventilation; $\dot{V}CO_2$: CO_2 production; VR: ventilation rate; $\dot{V}O_2$: oxygen uptake; HR: heart rate; $[lactate]_b$: blood lactate concentration; *P*: probability; NS: not significant.

the risk of triggering the polymerization/sickling cascade and vaso-occlusion. Indeed, the acidosis that accompanies substantial blood lactate accumulation,[26] triggers the polymerization/sickling cascade via a Bohr effect on the oxyhemoglobin dissociation curve.[4,27]

Endurance training improves metabolic flexibility

In the present study, RER values were elevated during rest and low-intensity exercise. This is not the first time that elevated RER values have been observed in SCD patients[6,13,28,29] and these values cannot be attributed to an unsteady state in the present study (see *Online Supplement*). A possible explanation is that patients with SCD may experience acid/base disturbances, as described by Maurel *et al.*,[30] who reported that 42% of SCD patients (stable and without renal failure) had baseline metabolic acidosis. Therefore, although high RER values would apparently indicate no or poor lipid oxidation, it cannot be excluded that lipid oxidation was partially masked by acid/base disturbances in the patients with SCD. In this context of high RER values, several data have been excluded (see Methods) to be able to assess substrate utilization and partitioning.

Nevertheless, considered collectively, the present results (RER values, %CHOox and %Lipidox, Table 2) suggest a high dependence on glycolytic sources in the energy supply at rest and during exercise in patients with SCD. In accordance, we have previously shown that skeletal muscle of patients with SCD has similar glycolytic but lower β-oxidation enzymes activities than control counterparts.[2] By extension, the present results suggest an apparent metabolic inflexibility[11] in patients with SCD.

Given the link between capillary density and glucose uptake[31] and the fact that patients with SCD have lower capillary density[2] as well as the links between insulin, hemodynamics and glucose uptake[32] and the observed hemodynamic disturbances in patients with SCD,[3,33] one could expect insulin resistance and lower glucose uptake in patients with SCD. Contrary to this hypothesis, insulin resistance and glucose uptake do not differ between SCD patients and control subjects.[34] Other studies even found lower insulin resistance[35] and higher insulin sensitivity[36] in SCD patients than in control subjects. From that point of view, the present results (elevated RER and %CHOox) suggest that glucose uptake and its subsequent utilization by the skeletal muscle are not dampened in patients with SCD. This latter inference is in accordance with the lower fasting blood glucose observed by Babiker *et al.*[37] Further studies would be necessary to characterize the relationship between this elevated glucose utilization and risk of metabolic syndrome in SCD; however, the prevalence of metabolic syndrome in sickle cell anemia patients has been reported to be approximately half of that in African-American counterparts.[38] Patients with SCD also seem to be less likely to develop obesity and diabetes mellitus compared to their peers.[39] As a whole, the lower insulin resistance[35] and fasting blood glucose[37] as well as the lower prevalence of metabolic syndrome, diabetes mellitus and obesity in SCD[38,39] are in agreement with the high glucose utilization found in the present study.

In healthy subjects, endurance training decreases utilization of carbohydrates (glycogen and glucose) and increases lipid oxidation for low-intensity exercises.[40-42] The trends towards lower post-training values of RER, CHOox and %CHOox along with higher Lipidox and %Lipidox during low-intensity exercise suggest that endurance training acts to some extent similarly in patients with SCD as in healthy subjects by improving metabolic flexibility. This training-induced beneficial adaptation is supported by the concomitant increase in activity of 3-hydoxylacyl-CoA dehydrogenase (a key enzyme involved in β-oxidation) in patients with SCD.[15] Of note, while endurance training appeared to improve metabolic flexibility in patients with SCD, the adaptations remained relatively modest. Further studies are necessary to determine the extent of benefits of a long-term endurance training program on substrate oxidation and partitioning in patients with SCD.

Post-exercise recovery

During active recovery, HR declined faster after training. In addition, VR decreased faster, $\dot{V}E$ decreased similarly and $\dot{V}E/\dot{V}CO_2$ increased less after training (Table 3). These latter results tend to support better ventilatory efficiency after training. The faster blood lactate decline observed during passive recovery is also in accordance with previous studies in healthy subjects.[16] Although fragmentary, the present results suggest that similar benefits of endurance training can be observed during post-exercise recovery in patients with SCD and in healthy subjects.

Experimental considerations and future directions

Classically, exercise-related physiological responses are evaluated using a maximal (symptom-limited) cardiopulmonary exercise test. While, several authors reported no complications (cardiac or other) during and after this type of exercise,[28,43,44] patients and physicians still have in mind that exercise may induce hemolysis[45] and that approximately one-third of vaso-occlusive crises and episodes of secondary acute chest syndrome are associated with exertion.[46] In this context, numerous patients and physicians remain reluctant to perform or prescribe a maximal (symptom-limited) cardiopulmonary exercise test, respectively. To convince patients and physicians that exercise testing may remain safe, we adopted a strategy using lactate concentration as a marker of safety. Lactate accumulation can testify the risk of triggering the polymerization/sickling cascade and vaso-occlusion through at least three mechanisms: metabolic acidosis, vasoconstriction, and cell adhesion (Figure 4). Acidosis that accompanies substantial blood lactate accumulation[26] triggers the polymerization/sickling cascade via a Bohr effect on the oxyhemoglobin

dissociation curve.[4,27] Second, lactate production is driven by muscle glycogenolysis, which is activated by adrenaline[47] due to progressive sympathetic nervous system activation with exercise intensity[17] (Figure 4). Of note, sympathetic nervous system activation induces vasoconstriction, and adrenaline activates cell adhesion via a cyclic adenosine monophosphate–dependent protein kinase A pathway,[48] both increasing the risk of hemodynamic disorders and potentially vaso-occlusion.[49-51] Given the potential implication of these mechanisms in the pathophysiology of SCD, avoiding rapid blood lactate accumulation may constitute an effective strategy of safety. For further information about the protocol/strategy used in the present study, we refer the reader to a previous paper.[4]

Complementary results of the present study should be highlighted. First, the lack of changes in some markers of hemolysis (lactate dehydrogenase, reticulocytes and total bilirubin) before and after training suggests that the proposed training program was not detrimental for the patients (Table 1). Second, all indices of patients' physical fitness ($W_{@LT1}$, $\dot{V}O_{2@LT1}$, $W_{@4mM}$, $\dot{V}O_{2@4mM}$ and $\%th\dot{V}O_{2peak@4mM}$) were improved in response to endurance training (Table 1). Although significant, the training-induced improvements observed in the present study were modest (Tables 1-3, Figures 1 and 3, *Online Supplementary Table S1*). Furthermore, because of high RER values, the number of available data to assess substrate partitioning and utilization was limited and the interpretation of metabolic changes (including metabolic flexibility) with endurance training should be strengthened by further investigations. As a whole, further studies including a larger number of patients with and without complications and a longer training period should allow a more precise assessment of the effects of endurance training in SCD patients.

It is important to keep in mind that the present results were obtained in patients without systemic complications, at steady state (see *Online Supplement*) and particularly without cardiovascular impairment. Indeed, cardiovascular complications are one of the leading causes of functional impairment and mortality in SCD.[52-54] Therefore, our results cannot be extended to more severely affected populations, which require dedicated trials that are currently underway. In patients with SCD, mitochondrial function is reduced compared to that of healthy HbAA counterparts[5] and is improved by endurance training.[15] Because mitochondrial respiration is believed to drive the oxygen uptake kinetics[55] (observed in everyday life when patients get up from a chair, climb stairs, etc.), it would be interesting in the near future to investigate oxygen uptake kinetics in SCD patients and the effects of endurance training on this kinetics. The expected post-training faster kinetics would further support the notion of improved oxygen delivery/uptake matching within skeletal muscles. Along the same line, because the improvement of mitochondrial function with endurance training is a central outcome in patients with SCD, any disturbances in mitochondrial function (e.g., *SOD2*^{V16A} variant[56]) which may dampen both the ability of patients to be physically active and improvements in response to endurance-training would deserve to be studied. Finally, it would be of great interest to investigate the effects of endurance training on nitric oxide bioavailability (which is known to be decreased in SCD patients[57] and constitutes a determinant factor of muscle oxygen supply during exercise[58]).

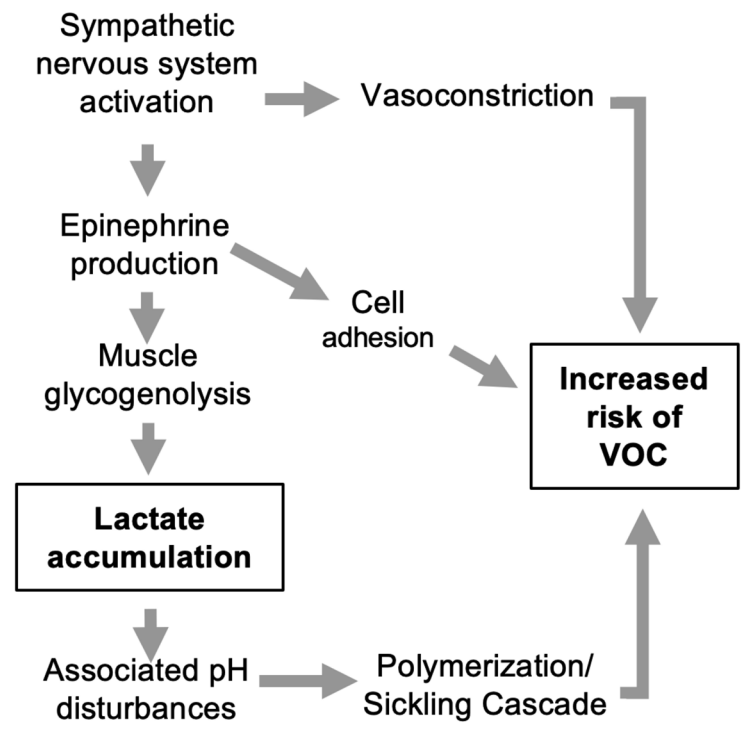

Figure 4. Lactate accumulation as a marker of safety. Lactate accumulation as a marker of (i) pH decrease triggering the polymerization/sickling cascade, and (ii) sympathetic nervous system activation and adrenaline production triggering vasoconstriction and cell adhesion, respectively, all of which ultimately lead to hemodynamic disturbances and potentially vaso-occlusive crises (VOC).

Conclusions

The main findings of the present study were that 8 weeks of endurance training in patients with SCD: (i) increased the slope coefficient of the $\dot{V}O_2$ *vs*. work rate relationship indicating a decrease of the oxygen supply/demand mismatch, (ii) blunted metabolic inflexibility, although the adaptations were modest and relied on a low number of data in the present study and (iii) improved some post-exercise cardiometabolic responses, as in the general population. As a whole, the present data reinforce the idea that endurance training is beneficial for patients with SCD.

Disclosures

PB has received grants from Addmedica, Fabre Foundation, Novartis and Bluebird in the past 36 months; has received consulting fees from Addmedica, Novartis, Roche, GBT, Bluebird, Emmaus, Hemanext, and Agios; has received honoraria for lectures from Novartis, Addmedica, and Jazzpharma; is a member of a Novartis steering committee; and is a co-founder of Innovhem.

Contributions

PB, BG, FG, LF, and LAM designed the study. AM, PB, BG, TR, FG, LF, and LAM performed the experiments and recorded the data. LM, MR, AM, LB, and LAM analyzed and interpreted the data. LM, MR, and LAM wrote the first draft. All authors critically revised and approved the present version of the manuscript. Artificial intelligence (AI) or AI-assisted technologies have not been used in the writing of this paper.

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This study is part of a larger experiment. A small part of the results presented here have been published elsewhere for other purposes.[8,14,15] The authors would like to thank all the patients for their interest and voluntary participation in the study.

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Data-sharing statement

Material described in the manuscript will be available for non-commercial purposes, without breaching participant confidentiality, and upon reasonable request by contacting the corresponding author.

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