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Original Article

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

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Short Title:

Endurance training in Sickle Cell Disease

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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 purpose[1–3]. The Authors would like to thank all the patients for their interest and voluntary participation in the study.

Authors' contribution

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. No AI or AI-assisted technologies have been used for the writing of the paper.

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Data sharing

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

Clinical trial

www.clinicaltrials.gov #NCT02571088

Abstract

Patients with sickle cell disease (SCD) display lower slope coefficients of the oxygen uptake ($V\dot{O}_2$) *vs.* work rate (W) relationship (delineating an O_2 uptake/demand mismatch) and a poor metabolic flexibility. Because endurance training (ET) increases the microvascular network and oxidative enzymes activity including one involved in lipid oxidation, ET might improve the slope coefficient of the $V\dot{O}_2$ *vs.* W curve and the metabolic flexibility of SCD patients. ET may also contribute to improve patient 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 ET. Minute ventilation, ventilation rate (VR), heart rate (HR), $V\dot{O}_2$, CO_2 production, respiratory exchange ratio, carbohydrate/lipid utilization and partitioning (including %Lipidox) and blood lactate concentration ($[lactate]_b$) were measured during and after SIT1 and SIT2. At baseline, the slope coefficient of the $V\dot{O}_2$ *vs.* W curve positively correlated with total hemoglobin, mean corpuscular hemoglobin and percentage of HbF. After training, the slope coefficient of the $V\dot{O}_2$ *vs.* W curve was significantly higher and the $[lactate]_b$ increase 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 VR, $V\dot{E}/V\dot{C}O_2$, HR and $[lactate]_b$ was faster after training. We concluded that ET 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.

Key words: incremental exercise; oxygen consumption; substrates partitioning; lactate; red blood cells

Introduction

Sickle cell disease (SCD) is the most common severe genetic disease and hemoglobinopathy in the world[4]. 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 the red blood cells (RBCs) a particular sickle shape. Sickle RBCs 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 sickle RBCs into 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[5] and an apparent tissue shunt[6] to limit oxygen transport and delivery deep into the tissues. The active skeletal muscle, which extensively uses oxygen for its energy metabolism, may particularly suffer from this impeded oxygen supply. Additionally, the muscle remodeling associated with SCD includes alterations of oxidative enzymes activity[5] indicative of impaired mitochondrial respiration. Therefore, if skeletal muscle is restricted in its oxygen supply, it is also limited in its capacity to consume it[5]. As a consequence, SCD patients display poor physical ability as well as particular/abnormal responses to physical activity[7,8]. A first particular response to exercise is the shape of the $\dot{V}O_2$ *vs.* work rate (W) relationship. Previous studies have shown that the slope of this relationship is lower in young adult[9] and adult[7,8] 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 the patients with SCD[10], delineating an oxygen uptake/demand mismatch[6]. A second particular response to exercise is the early and rapid blood lactate accumulation in patients with SCD[3,7]. If this early blood lactate accumulation signifies involvement of the glycolytic pathway in the energy supply, it also suggests a down-regulation in lipid utilization. Indeed, elevated lactate levels inhibit lipolysis[11] and function of carnitine palmitoyl transferase 1, the transporter of free fatty acids into mitochondria[12]. 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 lustrum, studies have investigated the potentially beneficial effects of moderate-intensity endurance-exercise training programs in patients with SCD[1,7,14,15]. At the muscular level, this type of training enlarged the microvascular network and improved the activity of key mitochondrial energetic metabolism enzymes[1,2]. 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 $\dot{V}O_2$ *vs.* W relationship, remains unknown. Furthermore, the well-documented blunting effect of endurance training on blood lactate concentrations in healthy subjects[16,17] occurs in patients with SCD[1,2], suggesting that the lactate-related inhibition on lipolysis and free fatty acid entry in mitochondria may also be partially blunted. In that 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[2]. 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

on cardio-respiratory parameters such as heart rate, minute ventilation and $V\dot{O}_2$, as well as on metabolic responses like blood lactate concentrations[16,18]. For the cardio-respiratory 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.

Therefore, the aim of the present study was 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 $V\dot{O}_2$ *vs.* W curve, ii) metabolic flexibility, and iii) post-exercise cardiopulmonary responses.

Methods

Study Population

Fifteen adult patients with homozygous SCD [HbSS or HbS/β0-thalassaemia genotypes; 7 women (2 took hydroxyurea) and 8 men (7 took hydroxyurea)] without severe chronic complications (see supplemental) participated in this study which took place in the Referral Centre for Major Sickle Cell Syndrome in Créteil, France. They received no transfusion or supplemental oxygen during the whole duration of the study, nor were they hospitalized for vaso-occlusive crisis. The study was approved by the ethics committee (Comité de Protection des Personnes Sud-Est 1 2014-14; EudraCT ID RCB 2014-A00334-43), in accordance with the Declaration of Helsinki (www.clinicaltrials.gov #NCT02571088). Volunteers were informed of the purposes, procedures, and possible associated risks and/or discomforts related to the protocol

before they gave written informed consent. Part of the results have been previously published for other purposes[1–3]. When necessary, they are repeated here for the convenience of readers.

Study design

Patients were subjected to blood samples (for complete blood count, HbS and HbF proportions, β-thalassemia, LDH 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 only 10 or 15 W for women or men, respectively. Gas exchange measurements [including ventilation rate (VR, cycle·min^{-1}), minute ventilation ($\dot{V}E$, 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 heart rate (HR, beats·min^{-1}) were continuously recorded. Every minute, whole blood lactate concentration ([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]$_b \geq 4$ mmol·L^{-1} was recorded[1,7]. 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 patient observation period ended when experimenters observed both i) a clear decrease in [lactate]$_b$ and ii) a value of [lactate]$_b$ below 4 mmol·L^{-1}. This session was used to determine (*vide infra*) i) indices of physical fitness, ii) the $\dot{V}O_2$ *vs.* 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 $mmol \cdot L^{-1}$. According to the $[lactate]_b$ obtained during each training session, exercise work rate for the subsequent training session was adjusted according to the strategy previously proposed[7]. A physician was present for the clinical observation of patients during each training session.

Blood lactate curve analysis

The blood lactate vs. 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 $mmol \cdot L^{-1}$ [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 $mmol \cdot L^{-1}$)[7] while work rate at 4 $mmol \cdot L^{-1}$ of [lactate]b was used as criterion for exercise termination.

Cardiopulmonary and gas exchange measurements and analyses

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

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

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

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

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

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

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

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

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

$$\%Lipidox\ (\%) = (100 - \%CHOox) \quad \text{(Eq. 7)}$$

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[13]. For RER values ≥ 1.0, Lipidox and %Lipidox were considered to be null.

Theoretical peak oxygen uptake and related parameters

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

$thV\dot{O}_{2peak} = 79.9 - (0.39 \cdot age) – (13.7 \cdot gender) – (0.127 \cdot weight)$ (Eq. 1).

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

Recovery data analysis

Different variables (VR, $V\dot{E}$, $V\dot{O}_2$, 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 performed 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 $\alpha = 0.05$.

Results

Patients' characteristics

Some patients' baseline and post-training characteristics are reported in Table 1. Hemoglobin concentration and indirect markers of hemolysis (LDH, 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 $V\dot{O}_2$ at LT1 and at exercise completion ($W_{@LT1}$, $V\dot{O}_{2@LT1}$, $W_{@4mM}$ and $V\dot{O}_{2@4mM}$, respectively) as well as $\%thV\dot{O}_{2peak@4mM}$ were all significantly improved by endurance training.

Applying Eq. 1 to the individual experimental data, mean ± SE r^2 value of the $V\dot{O}_2$ vs. 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 i) positively correlated with the hemoglobin concentration (Hb), the mean corpuscular hemoglobin (MCH) content and the

percentage of fetal hemoglobin (%HbF), as well as ii) negatively correlated with the percentage of hemoglobin S (%HbS, Figure 2). After training, the slope coefficient was correlated with Hb (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 V□E, V□O_2 and V□CO_2. During passive recovery (Δ0-6), 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, 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 4^{th} and 6^{th} minutes of passive recovery were lower after training ($p = 0.05$ and $p = 0.01$, respectively).

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 $V\dot{O}_2$ *vs.* 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 $V\dot{O}_2$ *vs.* W relationship has been reported to be lower in young adult and adult patients with SCD than in control subjects[7–9]. This lower slope coefficient suggests an oxygen uptake/demand mismatch resulting from lower muscle oxygen supply (supplemental) due to anemia, microvasculature rarefaction and low capillary/fiber surface of exchange, and/or lower muscle ability to consume oxygen (supplemental) as testified by the lower oxidative enzymes activity in SCD patients[5]. In accordance with this inference of an oxygen uptake/demand mismatch, blood lactate levels increased early (for very low exercise intensity) during incremental exercise in patients with SCD[7]. 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 extraction and testified by arterialization of venous blood in patients with SCD[6,24]. Interestingly, in the present study, the baseline slope coefficients were positively correlated with Hb, MCH and %HbF, as well as negatively correlated with %HbS (Figure 2, panels A, 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 indexes of the pathology.

Because endurance training enlarges the microvasculature, increases the capillary/fiber surface of exchange, and enhances oxidative enzymes activity (in other words, mitochondrial respiration) in patients with SCD[2,3], 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 $\dot{V}O_2$ *vs.* 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 reminds of results obtained in trained athletes. Indeed, Lacour *et al.* showed that the most successful athletes displayed a higher slope coefficient of the $\dot{V}O_2$ *vs.* W relationship associated with delayed blood lactate accumulation[25]. Of note i) that the observed increase in the slope coefficient after training was independent of any change in anemia since Hb concentration was not altered by endurance training, and ii) that after training, the correlation between the slope coefficients and Hb 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 indexes 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. Actually, this adaptation promotes better physical ability allowing patients with SCD to perform more vigorous activities of everyday life (e.g., climbing stairs, holding loads, walking faster)[3]. The concomitant delay in blood lactate accumulation is equally

significant because it should dampen the risk of triggering the polymerization/sickling cascade and vaso-occlusion. Indeed, acidosis that accompanies substantial blood lactate accumulation[26], triggers the polymerization/sickling cascade via a Bohr effect on the oxyhemoglobin dissociation curve [extensively discussed in previous papers[7,27]].

Endurance training improves metabolic flexibility

In the present study, RER values were elevated during rest and low exercise intensities. This is not the first time that elevated RER values have been observed in SCD patients[9,15,28,29] and these values cannot be attributed to an unsteady state in the present study (see supplemental). 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 (stable and without renal failure) SCD patients encountered baseline metabolic acidosis. Therefore, although high RER values would apparently indicate no or poor lipid oxidation, one may not exclude that lipid oxidation was partially masked by acid/base disturbances in patients with SCD. In that context of high RER values, several data have been excluded (see methods) to be able to assess substrate utilization and partitioning.

Nevertheless, in the aggregate, the present results (RER values, %CHOox and %Lipidox, Table 2) suggest a high dependence from 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 have similar glycolytic but lower β-oxidation enzymes activities than control counterparts[5]. By extension, the present results suggest an apparent metabolic inflexibility[13] in patients with SCD.

Given i) the link between capillary density and glucose uptake[31] and the fact that patients with SCD have lower capillary density[5] as well as ii) the links between insulin, hemodynamics

and glucose uptake[32] and the observed hemodynamic disturbances in patients with SCD[6,33], one may 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 carbohydrate utilization (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[2]. 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, V□E decreased similarly and V□E/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 (CPET). While, several authors reported no (cardiac or other) complications during and after this type of exercise[28,43,44], patients and physicians have still in mind that exercise may induce hemolysis[45] and that approximately 1/3 of vaso-occlusive crises and secondary acute chest syndromes are associated with exertion[46]. In that context, numerous patients and physicians remain reluctant to perform or prescribe a maximal (symptom-limited) CPET, respectively. To convince those 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 [extensively discussed in previous papers[7,27]]. 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[7].

Complementary results of the present study should be highlighted. First, the lack of changes in some markers of hemolysis (LDH, reticulocytes and total bilirubin) before and after training, suggests that the proposed training program was not detrimental for the patients (Table 1). Second, all indexes of patients' physical fitness ($W_{@LT1}$, $V\dot{O}_{2@LT1}$, $W_{@4mM}$, $V\dot{O}_{2@4mM}$ and $\%thV\dot{O}_{2peak@4mM}$) have been improved in response to endurance training (Table 1).

Although significant, the training-induced improvements observed in the present study were modest (Tables 1, 2 and 3, Figures 1 and 3, Supplemental table 1). 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 have been obtained in patients without systemic complications, at steady state (see supplemental) 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 severe populations, requiring dedicated trials currently underway.

In patients with SCD, mitochondrial function is reduced compared to healthy HbAA counterparts[5] and is improved by endurance training[2]. Because mitochondrial respiration is believed to drive the oxygen uptake kinetics[55] (observed in the 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 O_2 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 i) the ability of patients to be physically active and ii) the 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 NO bioavailability (which is known to be decreased in SCD patients[57] and constitute a determinant factor of muscle oxygen supply during exercise[58]).

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 the metabolic inflexibility, though these adaptations are modest and rely on a low number of data in the present study and iii) improved some post-exercise cardio-metabolic responses as in the general population. As a

whole, the present data reinforce the idea that endurance training is beneficial for patients with SCD.

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Table 1: Some characteristics of patients (n = 15).

	Demography and anthropometry		
	SIT1	SIT2	
Age (yr)	34.7 ± 11.1	na	
Height (cm)	172.1 ± 10.5	na	
Body Mass (kg)	65.2 ± 11.8	nm	
BMI ($kg \cdot m^{-2}$)	22.8 ± 2.9	nm	
	Hematology		
	SIT1	SIT2	p
Hb ($g \cdot dL^{-1}$)	9.2 ± 1.4	9.2 ± 1.5	0.846
Hct (%)	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 ($g \cdot dL^{-1}$)	33.6 ± 1.2	33.9 ± 1.3	0.344
LDH ($Ul \cdot L^{-1}$)	398 ± 148	373 ± 143	0.153
Reticulocytes (%)	6.4 ± 2.6	6.3 ± 3.1	0.899
Total bilirubin ($\mu mol \cdot L^{-1}$)	38.1 ± 28.1	36.6 ± 22.8	0.783
	Physical fitness parameters		
	SIT1	SIT2	p
$W_{@LT1}$ (W)	38.5 ± 10.8	54.8 ± 14.5	< 0.001
$V\dot{O}_{2@LT1}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	10.4 ± 2.6	13.6 ± 3.3	< 0.001
$W_{@4mM}$ (W)	70.4 ± 16.2	77.6 ± 15.1	0.006
$V\dot{O}_{2@4mM}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	14.9 ± 3.5	17.1 ± 3.0	0.029
$thV\dot{O}_{2peak}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	41.7 ± 6.4	na	na
$\%thV\dot{O}_{2peak@4mM}$ (%)	36.1 ± 8.2	41.6 ± 7.9	0.015
n</n> 52% $thV\dot{O}_{2peak@4mM}$	15/0	13/2	na

Values are mean ± standard error. SIT1 and SIT2: submaximal incremental exercise tests 1 and 2, respectively; na: non applicant; nm = not measured. BMI: Body Mass Index; Hb: hemoglobin; Hct: hematocrit. HbS and F: hemoglobin S and F; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; LDH: lactate dehydrogenase; $W_{@LT1}$ and $V\dot{O}_{2@LT1}$: work rate and oxygen uptake at the first lactate threshold; $W_{@4mM}$ and $V\dot{O}_{2@4mM}$: work rate and $V\dot{O}_2$ at the 4 mM blood lactate concentration i.e., at exercise cessation; $thV\dot{O}_{2peak}$: theoretical peak oxygen uptake; $\%thV\dot{O}_{2peak@4mM}$: percentage of $thV\dot{O}_{2peak}$ at 4 mM of blood lactate concentration; n = number of patients; p = probability; NS: Not significant.

Table 2. Blood lactate concentrations, gas exchanges and substrates 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})	$V\dot{O}_2$ (L·min^{-1})	$V\dot{C}O_2$ (L·min^{-1})	RER	CHOox (g·min^{-1})	Lipidox (g·min^{-1})	%CHOox (%)	%Lipidox (%)
Rest		(n =15)	(n = 12)	(n = 12)	(n = 12)	(n = 12)	(n = 12)	(n = 12)	(n = 12)
	SIT1	1.7 ± 0.6	0.33 ± 0.1	0.37 ± 0.1	1.12 ± 0.2	0.3 ± 0.2	0.0 ± 0.0	99.7 ± 26.5	0.3 ± 26.5
	SIT2	1.6 ± 0.5	0.28 ± 0.1	0.26 ± 0.1	0.93 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	95.9 ± 31.6	4.1 ± 31.6
		NS	NS	NS	p = 0.03	NS	NS	NS	NS
20/30 W		(n = 14)	(n = 14)	(n = 14)	(n = 14)	(n = 14)	(n = 14)	(n = 14)	(n = 14)
	SIT1	1.7 ± 0.5	0.50 ± 0.1	0.55 ± 0.1	1.10 ± 0.1	0.6 ± 0.2	0.0 ± 0.1	88.5 ± 24.5	11.5 ± 24.5
	SIT2	1.6 ± 0.5	0.62 ± 0.1	0.56 ± 0.1	0.90 ± 0.1	0.5 ± 0.2	0.1 ± 0.1	74.5 ± 30.3	25.5 ± 30.3
		NS	NS	NS	p = 0.003	NS	NS	NS	NS
30/45 W		(n = 15)	(n = 11)	(n = 11)	(n = 11)	(n = 11)	(n = 11)	(n = 11)	(n = 11)
	SIT1	2.1 ± 0.6	0.74 ± 0.2	0.72 ± 0.2	0.97 ± 0.0	0.9 ± 0.3	0.0 ± 0.0	96.4 ± 11.2	3.6 ± 11.2
	SIT2	1.7 ± 0.4	0.78 ± 0.1	0.70 ± 0.1	0.90 ± 0.1	0.7 ± 0.3	0.1 ± 0.1	72.2 ± 30.1	27.8 ± 30.1
		p = 0.006	NS	NS	p = 0.04	NS	p = 0.04	p = 0.04	p = 0.04
40/60 W		(n = 15)	(n = 7)	(n = 7)	(n = 7)	(n = 7)	(n = 7)	(n = 7)	(n = 7)
	SIT1	2.7 ± 0.8	0.80 ± 0.2	0.83 ± 0.2	1.04 ± 0.2	0.9 ± 0.2	0.0 ± 0.1	93.7 ± 14.0	6.3 ± 14.0
	SIT2	2.1 ± 0.6	0.90 ± 0.2	0.81 ± 0.2	0.90 ± 0.1	0.8 ± 0.2	0.1 ± 0.1	69.4 ± 18.2	30.6 ± 18.2
		p = 0.005	NS	NS	p = 0.01	NS	NS	p = 0.02	p = 0.02

Values are mean ± standard error. RER: respiratory exchange ratio; CHOox: Carbohydrate oxidation; Lipidox: Lipid oxidation; %CHOox and %Lipidox: substrate partitioning. p = probability; NS: Not significant.

Table 3. Time courses of V□E, V□E/V□CO_2, VR, V□O_2, HR 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**
V□E ($L \cdot min^{-1}$)	SIT1	52.9 ± 7.6	35.5 ± 5.8	-17.4 ± 7.9	31.2 ± 4.6	19.4 ± 4.8	17.1 ± 4.4	17.1 ± 3.2	-34.7 ± 5.1
	SIT2	53.0 ± 6.7	34.3 ± 5.7	-18.7 ± 6.5	32.0 ± 5.9	18.9 ± 4.4	16.8 ± 4.5	16.7 ± 4.7	-36.5 ± 4.8
		NS	NS	NS	NS	NS	NS	NS	NS
V□E/V□CO_2	SIT1	41.8 ± 6.3	44.9 ± 6.9	3.1 ± 3.1	43.7 ± 6.0	47.4 ± 6.4	49.8 ± 4.2	50.2 ± 3.5	1.0 ± 18.3
	SIT2	39.4 ± 6.1	41.7 ± 6.2	2.3 ± 2.4	42.3 ± 6.6	48.2 ± 7.3	50.0 ± 5.7	53.5 ± 6.5	5.1 ± 20.3
		NS	NS	p = 0.001	NS	NS	NS	NS	NS
VR ($cycle \cdot min^{-1}$)	SIT1	39.7 ± 11.5	35.4 ± 11.4	- 4.4 ± 6.6	30.6 ± 8.6	24.1 ± 4.6	21.5 ± 4.6	21.0 ± 4.2	-15.0 ± 5.5
	SIT2	37.2 ± 7.2	30.0 ± 6.8	-7.2 ± 4.3	28.8 ± 6.0	23.0 ± 4.3	21.4 ± 3.9	22.4 ± 4.02	-14.1 ± 4.7
		NS	p = 0.04	NS	NS	NS	NS	NS	NS
V□O_2 ($L \cdot min^{-1}$)	SIT1	1.0 ± 0.2	0.7 ± 0.2	-0.3 ± 0.2	0.6 ± 0.2	0.4 ± 0.4	0.3 ± 0.1	0.3 ± 0.1	-0.6 ± 0.1
	SIT2	1.1 ± 0.3	0.7 ± 0.2	-0.4 ± 0.2	0.7 ± 0.2	0.6 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	-0.8 ± 0.2
		NS	NS	NS	NS	NS	NS	NS	p = 0.02
HR ($beats \cdot min^{-1}$)	SIT1	156.0 ± 14.4	131.6 ± 14.7	-24.4 ± 8.4	126.5 ± 14.4	103.6 ± 10	97.5 ± 10.4	97.4 ± 9.3	-28.0 ± 12.1
	SIT2	154.1 ± 16.2	124.5 ± 10.1	-29.6 ± 7.5	121.6 ± 9.8	99.5 ± 8.4	95.7 ± 9.3	94.9± 10.4	-24.7 ± 9.2
		NS	p = 0.02	p = 0.02	NS	NS	NS	NS	NS
$[lactate]_b$ ($mmol \cdot L^{-1}$)	SIT1	4.8 ± 0.6	4.9 ± 0.6	0.0 ± 0.4	4.8 ± 0.7	4.5 ± 0.5	4.1 ± 0.6	4.1 ± 0.4	-0.7 ± 0.4
	SIT2	5.0 ± 0.6	5.0 ± 0.6	-0.0 ± 0.2	5.0 ± 0.6	4.6 ± 0.6	4.2 ± 0.8	3.9 ± 0.5	-1.3 ± 0.5
		NS	NS	NS	NS	NS	p = 0.05	p = 0.01	p = 0.01

Values are mean ± standard error. V□E: minute ventilation; V□CO_2: CO_2 production; VR: ventilation rate; V□O_2: oxygen uptake; HR: Heart Rate; $[lactate]_b$: blood lactate concentration; p = probability; NS: Not significant.

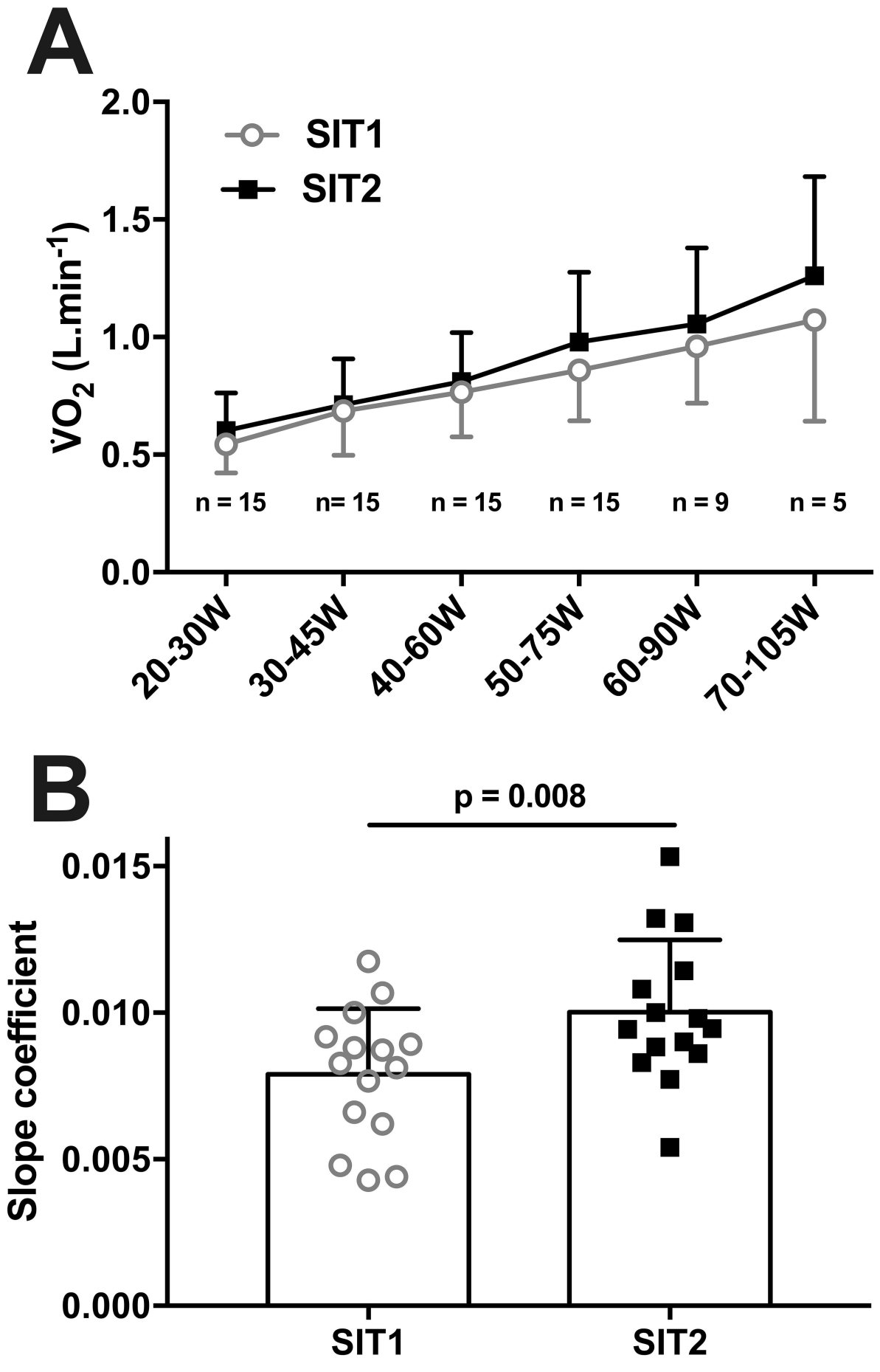
Figure legends

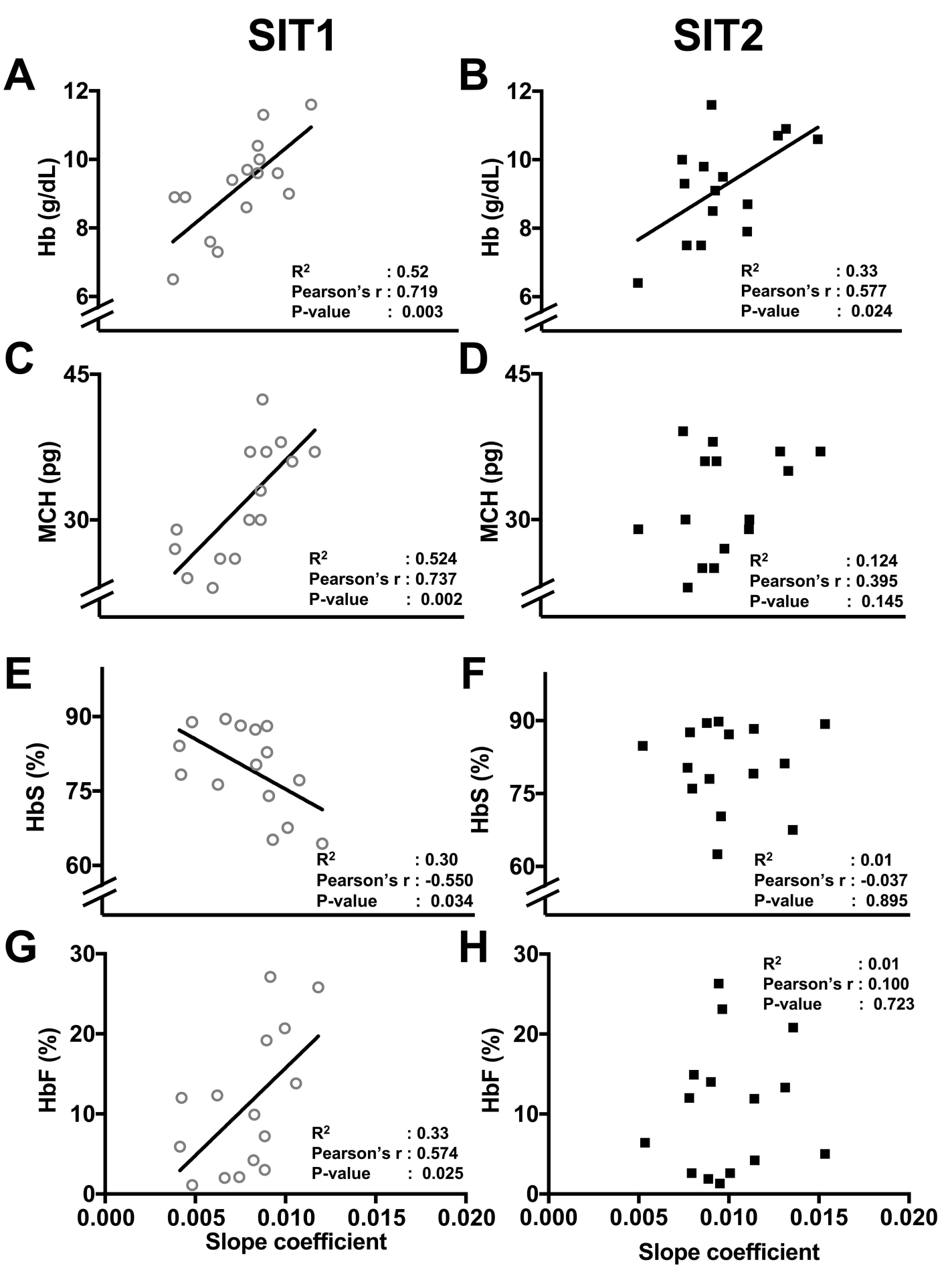
Figure 1. $V\dot{O}_2$ and work rate relationships. A: Mean ± SD $V\dot{O}_2$ *vs.* work rate curves before (SIT1) and after (SIT2) training. B: Slope coefficients of the $V\dot{O}_2$ *vs.* work rate relationship before (SIT1) and after (SIT2) training. Open dots and black squares are mean or individual values before and after training, respectively. SIT: submaximal incremental test.

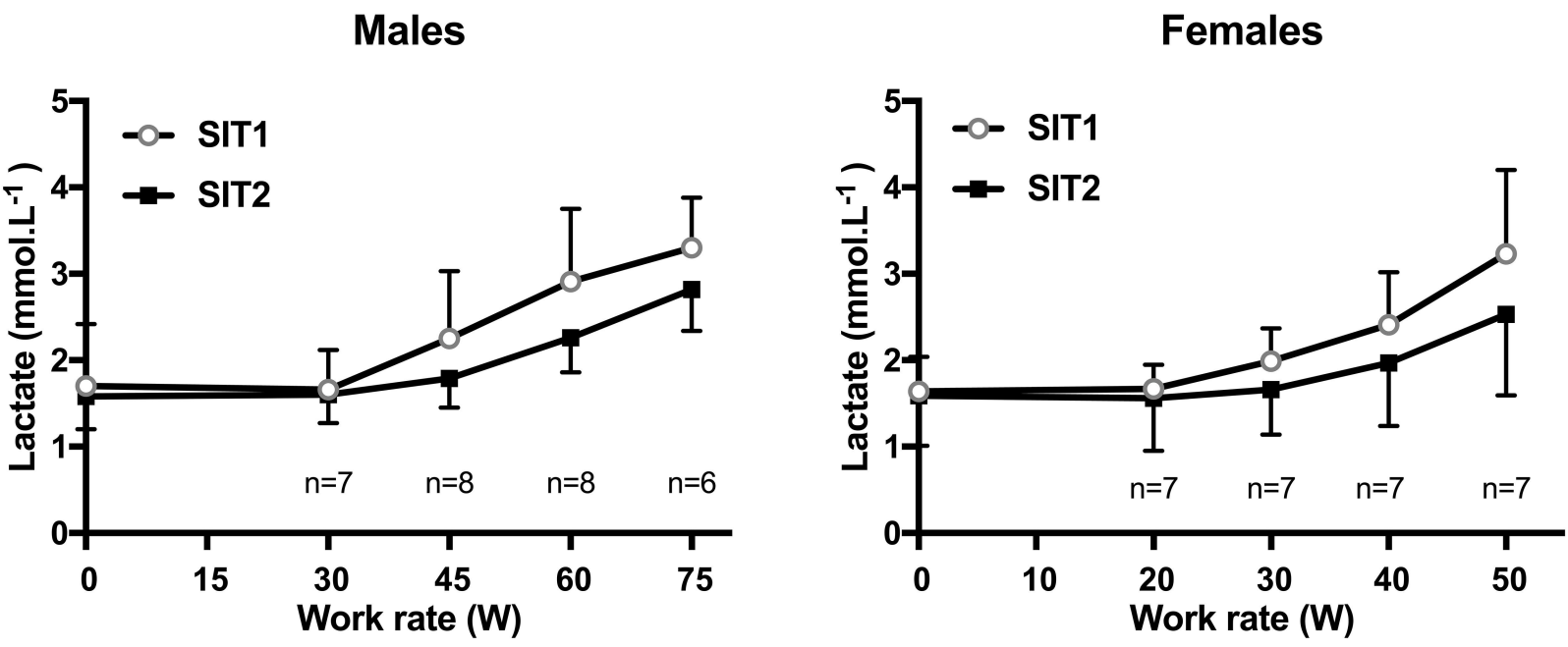
Figure 2. Correlations between the slope coefficient of the $V\dot{O}_2$ *vs.* work rate curves and total hemoglobin, mean corpuscular hemoglobin, percentage of HbS and percentage of HbF obtained before (SIT1) and after (SIT2) training. SIT: submaximal incremental test.

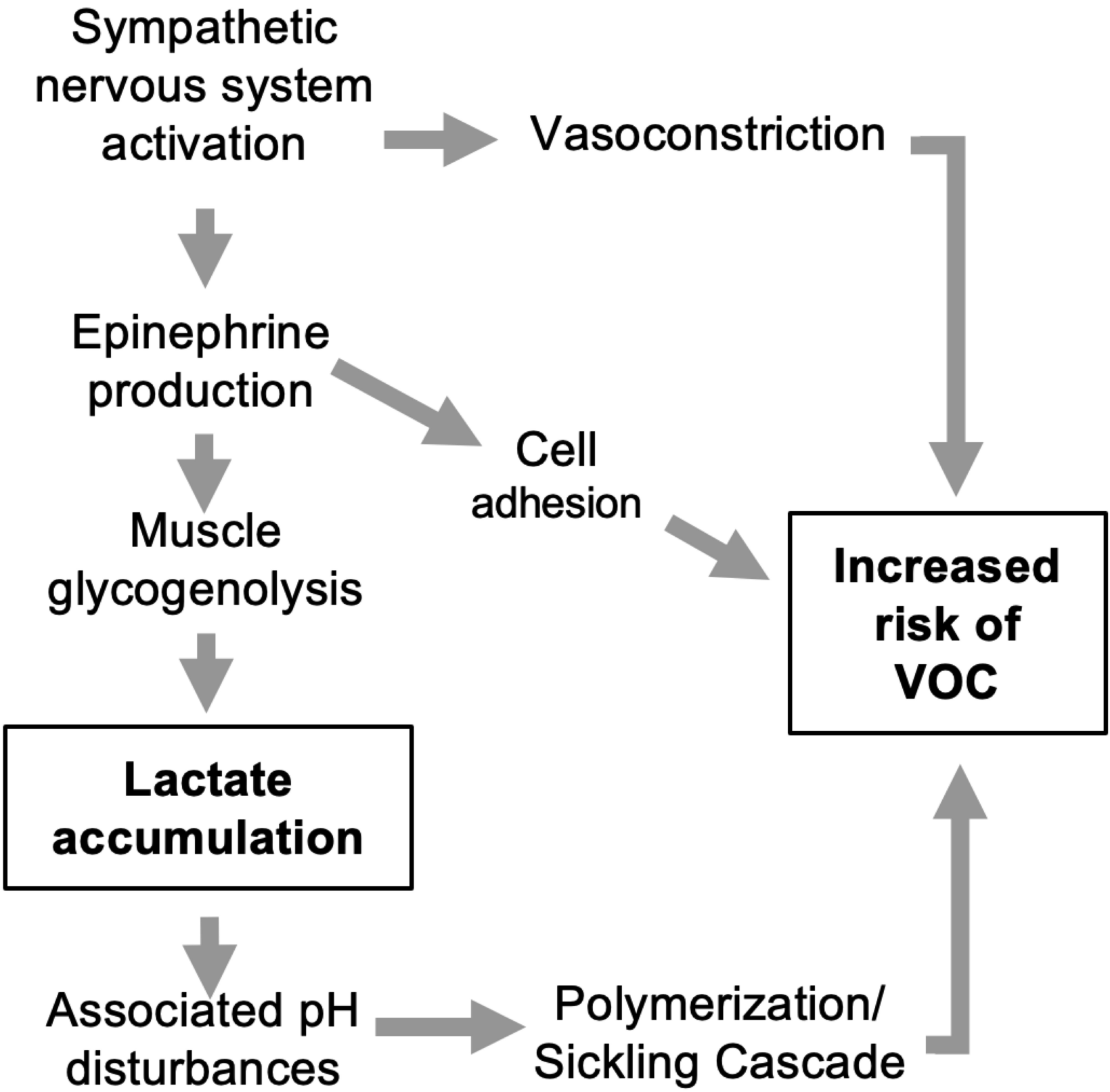
Figure 3. Blood lactate concentrations as a function of power output in males and females during the submaximal incremental test before (SIT1) and after (SIT2) endurance training.

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 (SNS) activation and adrenaline production triggering vasoconstriction and cell adhesion, respectively, all of which ultimately leading to hemodynamic disturbances and potentially vaso-occlusive crisis (VOC).


A
2.0
1.5
1.0
0.5
0.0
SIT1
SIT2
$\dot{V}O_2$ (L.min^{-1})
n = 15
n= 15
n = 15
n = 15
n = 9
n = 5
20-30W
30-45W
40-60W
50-75W
60-90W
70-105W
B
p = 0.008
0.015
0.010
0.005
0.000
Slope coefficient
SIT1
SIT2


SIT1
SIT2
A
B
C
D
E
F
G
H
Hb (g/dL)
MCH (pg)
HbS (%)
HbF (%)
Slope coefficient
R2 : 0.52
Pearson's r : 0.719
P-value : 0.003
R2 : 0.33
Pearson's r : 0.577
P-value : 0.024
R2 : 0.524
Pearson's r : 0.737
P-value : 0.002
R2 : 0.124
Pearson's r : 0.395
P-value : 0.145
R2 : 0.30
Pearson's r : -0.550
P-value : 0.034
R2 : 0.01
Pearson's r : -0.037
P-value : 0.895
R2 : 0.33
Pearson's r : 0.574
P-value : 0.025
R2 : 0.01
Pearson's r : 0.100
P-value : 0.723


Males
SIT1
SIT2
Lactate (mmol.L-1)
0
1
2
3
4
5
n=7
n=8
n=8
n=6
0
15
30
45
60
75
Work rate (W)
Females
SIT1
SIT2
Lactate (mmol.L-1)
0
1
2
3
4
5
n=7
n=7
n=7
n=7
0
10
20
30
40
50
Work rate (W)


Sympathetic nervous system activation
Vasoconstriction
Epinephrine production
Cell adhesion
Muscle glycogenolysis
Increased risk of VOC
Lactate accumulation
Associated pH disturbances
Polymerization/ Sickling Cascade

Supplemental

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

Mougin *et al.*

Supplemental information on the population of patients

None of the patients displayed severe complications including chronic inflammatory or infectious disease, kidney insufficiency, clinical signs and/or history of heart failure, left ventricular ejection fraction <50%, pulmonary arterial hypertension with tricuspid regurgitation velocity >2.8 m/s, atrial fibrillation, ventricular arrhythmias, left ventricular hypertrophy, significant valvulopathy, known coronary disease, uncontrolled hypertension, treatment with anti-arrhythmia drugs (including β-blockers), current anti-coagulant treatment, a pacemaker or defibrillator, known cerebral vasculopathy, prior stroke and epilepsy.

Supplemental results and discussion

The slope coefficient of the $\dot{V}O_2$ *vs.* W relationship has been reported to be lower in young adult and adult patients with SCD than in control subjects[1–3]. This lower slope coefficient likely results from lower muscle oxygen supply (due to anaemia, microvasculature rarefaction and low capillary/fibre surface of exchange) and/or lower muscle ability to consume oxygen (as testified by

lower oxidative enzymes activity, lower peripheral extraction and arterialisation of venous blood) in SCD patients[4–6]. These results and inferences are in total accordance with a previous study performed in patients with COVID-19 for whom the observed lower slope coefficient of the $\dot{V}O_2$ *vs.* W relationship was concomitant to both lower arterial oxygen content and peripheral extraction during rest as well as exercise[7].

In the present study, we recorded elevated RER values during rest and low-intensity exercise. Firstly, as mentioned in the main text, this is not the first time that elevated RER values are reported in SCD patients[1,8–10]. Second, these values cannot be related to metabolic cart dysfunction. Indeed, to ensure validity of gas exchange measurements, the metabolic cart underwent standard service before the study and at mid experiments. Furthermore, the metabolic cart underwent successful calibration before each measurement session. Additionally, the experiments took place over 18 months, during which time values remained similar. Furthermore, it cannot be argued that data are related to unsteady states. Supplemental figure 1 shows clearly that a steady state in oxygen uptake is reached during the last 10 breathing cycles of each stage of the submaximal incremental tests (SIT1 and SIT2). This is actually not surprising. Indeed, the increment between two successive stages is so small (10 W for women and 15 W for men) that even if the stage duration is rather short (2 min), a steady state is reached. Along the same line of reasoning, the steady state being obtained before and after training it cannot be argued that the improvement of the slope coefficient is false/fake and related to an unsteady state before training and a steady state after training. In the aggregate, the proper functioning of the metabolic cart and the steady states obtained during the last 20 seconds of each stage during SIT1 and SIT2 argue in favor of the facts that i) the RER values were correct, strongly suggesting acid/base disturbances in patients with SCD, ii) the change of the slope coefficient was real and not due to unsteady states before training and steady states

after training, and iii) when possible/appropriate, substrate partitioning calculation were reliable, strongly suggesting a predominantly carbohydrate utilization in patients with SCD.

The supplemental table 1 reports metabolic equivalent of task (MET) values for each patient during SIT1 as SIT2.

References

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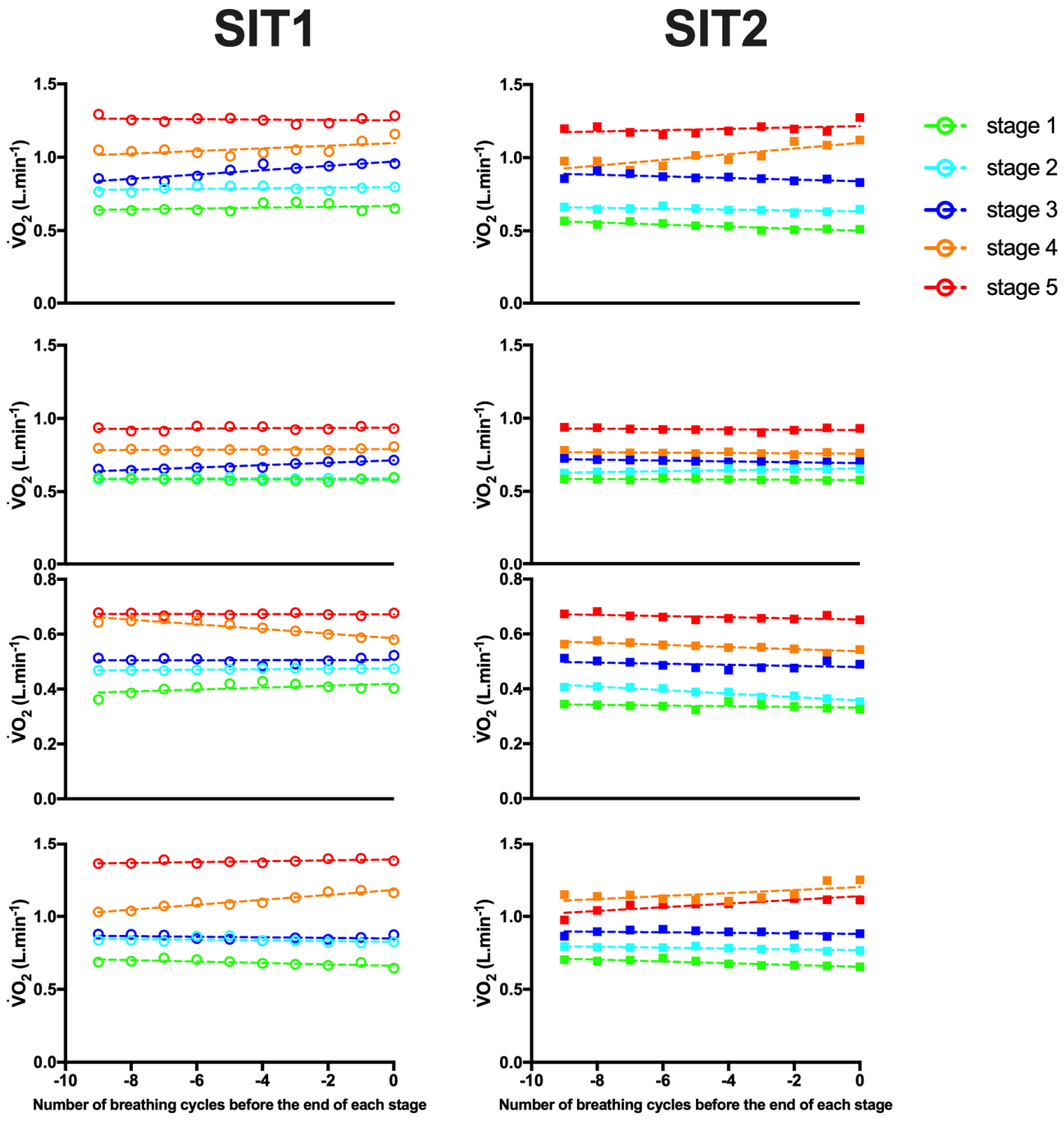
Supplemental table 1: Metabolic equivalent of task (MET) for each step of SIT1 and SIT2.

Males		30 W	45 W	60 W	75 W	90 W	105 W	120 W	135 W
1	SIT1	2.09	2.58	3.54	4.19	4.51			
	SIT2	2.74	3.18	3.74	4.19	4.95			
2	SIT1	2.36	4.02	3.95	4.32	4.75	5.95		
	SIT2	2.56	3.06	3.09	4.29	4.75	5.91		
3	SIT1	2.17	2.42	3.00					
	SIT2	1.63	2.24	2.71	*3.58*				
4	SIT1	1.72	2.77	3.24	3.95	4.45	*4.62*		
	SIT2	3.95	4.37	4.79	5.55	6.64	*6.60*		
5	SIT1	3.04	3.66	3.84	4.82	6.12			
	SIT2	2.72	3.53	3.93	5.00	*4.46*			
6	SIT1	2.65	3.35	4.04	4.24	*4.37*			
	SIT2	3.27	3.96	4.65	6.16	6.16			
7	SIT1	1.82	2.86	2.94	*3.55*				
	SIT2	2.99	3.72	4.55	5.54	5.54			
8	SIT1	2.37	3.35	3.67	4.04				
	SIT2	2.78	3.43	3.63	4.29	4.24	4.82	5.10	
Females		20 W	30 W	40 W	50 W	60 W	70 W	80 W	90 W
1	SIT1	1.78	2.20	2.34	2.95	3.19	3.23		
	SIT2	1.78	1.87	2.34	2.62	3.09	3.89	4.26	
2	SIT1	2.91	3.29	3.45	3.61	3.83	4.37	*4.91*	
	SIT2	2.16	2.53	3.29	3.56	4.26	4.64	5.07	5.23
3	SIT1	2.39	2.75	3.64	3.90	*4.42*			
	SIT2	2.55	2.86	3.27	4.00	*4.52*			
4	SIT1	2.55	2.62	2.86	2.62	3.10	3.34		
	SIT2	2.10	2.58	2.79	3.10	3.34	4.34	*4.72*	
5	SIT1	1.92	2.36	2.47	2.69				
	SIT2	2.42	2.58	2.75	3.35	*3.79*			
6	SIT1	3.38	3.44	3.91	4.49	5.42			
	SIT2	3.38	3.73	4.20	4.78	5.36			
7	SIT1	3.03	3.31	3.42	4.15				
	SIT2	2.91	3.31	3.75	4.31	4.59			

Values on the left hand side, in italic and grey were obtained at minute one of the two-minute stage.

Figure legends

Supplemental figure 1. Typical $\dot{V}O_2$ responses during the last 10 breathing cycles of each stage of SIT1 (before training, left panel) and SIT2 (after training, left panel) in four typical subjects (two males and two females). $\dot{V}O_2$ is a wavelet mean value over 5 seconds. SIT: submaximal incremental exercise test.


SIT1
SIT2
stage 1
stage 2
stage 3
stage 4
stage 5
V̇O2 (L.min-1)
Number of breathing cycles before the end of each stage