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# Red blood cell deformability and aggregation, cell adhesion molecules, oxidative stress and nitric oxide markers after a short term, submaximal, exercise in sickle cell trait carriers

Julien Tripette<sup>a,b</sup>, Philippe Connes<sup>a</sup>, Éric Beltan<sup>c</sup>, Tawfik Chalabi<sup>c</sup>, Laurent Marlin<sup>a</sup>, Roger Choutc<sup>c</sup>, Oguz K Baskurt<sup>d</sup>, Olivier Hue<sup>a</sup> and Marie-Dominique Hardy-Dessources<sup>b,\*</sup>

<sup>a</sup>Laboratoire ACTES (EA 3596), Département de Physiologie, Université des Antilles et de la Guyane, Pointe-à-Pitre, France

<sup>b</sup>Inserm U763, Université des Antilles et de la Guyane, Pointe-à-Pitre, France

<sup>c</sup>Service d'Hématologie, CHU de Pointe-à-Pitre, France

<sup>d</sup>Department of Physiology, Akdeniz University Faculty of Medicine, Antalya, Turkey

**Abstract.** The present study investigated the hemorheological and endothelial alterations in sickle cell trait (SCT) carriers in response to a submaximal exercise. Eleven SCT carriers and 11 subjects with normal hemoglobin performed submaximal exercise for 15 min. Blood was sampled at rest, at the end of exercise, and at 2 and 24 h of recovery. Hemorheological alterations observed in the SCT group were as follows: 1) lower RBC deformability at high shear stress at all time-points, with no relation to oxidative stress, 2) higher disaggregation threshold at all time-points, suggesting RBC hyper-aggregation, and 3) higher blood viscosity at the end of exercise and during recovery. Exercise had a specific influence on the levels of the soluble cell adhesion molecules P and L-selectin in the SCT carriers, with higher P-selectin levels at all time-points and a greater increase in L-selectin levels during recovery. SCT carriers had slightly decreased nitrite levels at 24 h of recovery, which might be clinically insignificant. In conclusion, the hemorheological alterations in association with lower NO production found in the SCT carriers are probably not sufficient to explain the medical complications sometimes reported in SCT carriers after exercise.

Keywords: Blood rheology, inflammation, endothelium, submaximal

## 1. Introduction

Sickle cell anemia is a genetic hemoglobinopathy characterized by chronic hemolysis and recurrent painful vaso-occlusive crises, with marked variation in the severity of symptoms among individuals. The vaso-occlusive crises result from both physical obstruction of small blood vessels by rigid sickle-shaped red blood cells (RBC) and vascular adhesion of circulating cells on the endothelial wall [9].

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\*Corresponding author: Dr. Marie-Dominique Hardy-Dessources, UMR-Inserm 763, Université des Antilles et de la Guyane, Hôpital Ricou – CHU Pointe-à-Pitre, 97159 Pointe-à-Pitre, Guadeloupe (French West Indies), France. E-mail: marie-dominique.hardy-dessources@inserm.fr.

Sickle cell trait (SCT) is the heterozygous form of sickle cell anemia. SCT carriers have both hemoglobin (Hb) A and HbS, and they are usually considered as asymptomatic. However, recent reports pointed out a possible relationship with sudden death events [10] and significantly increased morbidity among SCT carriers [33]. Several studies reported cases of exercise-related sudden death [15, 16, 18, 37] in SCT carriers. Kark et al. [16] observed a 30-fold higher rate of unexplained exercise-related sudden death among black recruits with SCT compared with those carrying normal Hb. In addition, some authors proposed that SCT should be reclassified as a co-factor for morbidity when other pathologies like diabetes are present [1], while others strongly suggested that SCT might be a risk factor for coronary artery disease and venous thromboembolism [2]. Exercise induces several changes, such as hypoxia, acidosis, dehydration, hyperosmolality and hyperthermia, which might promote polymerization of HbS [17]. Moreover, some data suggest a possible role of the exercise-induced stress/inflammatory response in the deleterious events reported in SCT carriers, in addition to hemorheological changes [11]. Altogether, the metabolic changes and the specific hemorheological and immune responses induced by exercise could transform silent SCT into a syndrome resembling sickle cell disease with vaso-occlusive crises.

Our recent studies described hemorheological alterations and vascular abnormalities in SCT carriers in response to intense/heavy exercise [11, 20, 21, 31, 32]. Monchanin et al. [21] and Tripette et al. [31] observed differences in the kinetics of sVCAM-1 and/or selectins in response to a progressive and maximal exercise, which may have deleterious effects on blood flow by promoting abnormal adherence between the circulating cells and the endothelial cells. In addition, SCT carriers presented higher blood viscosity ( $\eta_b$ ) and a delayed alteration in RBC deformability compared with subjects with normal hemoglobin after maximal/strenuous exercise [20, 32]. The reasons for these delayed hemorheological alterations in SCT carriers are unknown but the results obtained by Senturk et al. [24] in healthy normal subjects suggested that late alterations in RBC deformability after exercise might be related to oxidative stress. These alterations are generally thought to affect blood flow in the microcirculation, but vascular autoregulatory mechanisms of the cardiovascular system can easily compensate for a certain degree of mechanical RBC alteration if sufficient vasodilatory reserve exists [5]. It remains unknown whether the nitric oxide (NO) production is normal or not in SCT carriers.

The medical complications observed in SCT carriers have not been reported only in response to intense or heavy exercise; several authors reported exercise-related sudden death in response to moderate efforts [17, 18, 37]. In order to complete our previous investigations, the present study was planned with the following aims: 1) to attain a better understanding of the mechanisms involved in post-exercise SCT complications with special interests on red blood cell aggregation and NO marker; 2) to investigate the effects of a submaximal/moderate exercise. We examined hematological and hemorheological parameters, plasma levels of several vascular adhesion molecules and different markers of endothelial function in both SCT and control subjects, in response to 15 min of submaximal exercise performed above the first ventilatory threshold. We also investigated markers of oxidative stress in relation to blood rheology.

## 2. Material and methods

### 2.1. Ethics information and written informed consent

The ethics committee of the Academic Hospital of Pointe-à-Pitre approved the present protocol. The subjects were informed of the procedures and purposes of the study, and they gave their written informed consent. The protocol was in accordance with the guidelines set by the Declaration of Helsinki.

## 2.2. Subjects

Eleven SCT carriers (SCT group) and 11 subjects of African descent with normal hemoglobin (CONT group) participated in the study. All subjects were students at the University of the French West Indies and Guiana. They reported regular training in sports activities (8 hr/week  $\pm$  2 hr) but had never practiced sports at a high level: they were moderately trained. In addition, none of them reported regular cycling practice. The major exclusion criteria included cardiovascular disorders, stroke, obesity and  $\alpha$ -thalassemia. None of the subjects presented a significant medical history and none was under medical treatment.

## 2.3. SCT and $\alpha$ -thalassemia diagnosis

Venous blood was drawn into tubes containing EDTA and screened by isoelectric focusing in order to test for the hemoglobin type. The results were confirmed by citrate agar electrophoresis. The various hemoglobins were isolated and quantified by high performance liquid chromatography (HPLC). A test of solubility confirmed the presence of HbS. Positive test results for SCT were determined by the presence of HbS (<50%) and a normal percentage of HbA2. Diagnosis of  $\alpha$ -thalassemia was made from both resting hematological parameters and the single-tube multiplex-PCR technique as already done elsewhere [20, 21, 30–32]. The mean concentration of HbS in the SCT group was  $39.0 \pm 0.4\%$ .

## 2.4. Protocol

Anytime during the two weeks preceding the main experimental exercise, the subjects performed a maximal and progressive exercise test to determine their maximal exercise responses, the first ventilatory threshold and aerobic fitness. A ramp exercise test was performed on an electromagnetically braked cycloergometer (Ergoline 500, Bosch, Germany). It began with a 2-min warm-up at 30 W, and the load was increased by 30 W every minute until exhaustion. Pedaling speed remained constant between 60 and 70 rpm during the entire test.

Gas exchanges were measured using a breath-by-breath automated exercise metabolic system (ZAN 600 USB, Oberthulba, Germany) until maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) was reached. A 12-lead electrocardiogram (ZAN 600 ECG, Oberthulba, Germany) was used to continuously monitor heart rate. The first ventilatory threshold (VT1) and the maximal aerobic power (MAP) were also determined. The VT1 was identified as the point of nonlinear increase in  $\dot{V}E$  and a clear increase in  $\dot{V}E/\dot{V}O_2$  [36]. At the same time,  $\dot{V}E/\dot{V}CO_2$  remained constant or slightly decreased. VT1 was also determined by the V-slope method of Beaver et al. [7].

The subjects did not participate in any physical activity during the two days preceding the day of main experiment. During the main experiment the subjects completed a 15-min submaximal exercise on the same electromagnetically braked cycloergometer (Ergoline 500, Bosch, Germany). The workload was set at 5% over the VT1 determined during the first progressive and maximal exercise test and was kept constant throughout the trial. Pedaling speed remained constant between 60 and 70 rpm during the entire exercise. A 12-lead electrocardiogram (ZAN 600 ECG, Oberthulba, Germany) continuously monitored the heart rate.

Venous blood samples were drawn from the antecubital vein of the left arm at rest ( $T_R$ ), at the end of exercise ( $T_{EX}$ ), after 2 h of recovery ( $T_{REC}$ ), and after 24 h of recovery ( $T_{24}$ ). The laboratory was air-conditioned (room temperature 23–25°C). No drink or food was allowed before, during and for 2 h after exercise. It was requested that subjects avoid exercise training for the whole period of recovery.

## 2.5. Hematological measurements

Total counts of leukocytes, polynuclear neutrophils, lymphocytes, monocytes, platelets, RBC, and reticulocytes (expressed in percentages), hemoglobin concentration ([Hb]), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were determined from blood sampled in EDTA tubes at  $T_R$ ,  $T_{Ex}$ ,  $T_{Rec}$  and  $T_{24}$ , using a hematology analyzer (Max M-Retic, Coulter, USA). Hematocrit (Hct) was measured by a micro-method after blood microcentrifugation (12,000g, 10 min, 25°C) (HEMA-C, Jouan, France).

Fibrinogen ( $C_F$ , g/L) measurement was performed on a Behring Coagulation System (Dade Behring, Germany) using Multifibren U (thrombin solution) reagent. Quantitative determination of von Willebrand factor activity was performed by turbidimetric assay (von Willebrand Ag Test Kit, Dade Behring, Germany).

## 2.6. Adhesion molecule measurements

Blood was collected in heparin tubes at every time-point ( $T_R$ ,  $T_{Ex}$ ,  $T_{Rec}$ ,  $T_{24}$ ) and plasma was separated by centrifugation (1000g, 4°C, 5 min) and stored at -80°C for subsequent analysis. ELISA kits were used to assay the plasma concentrations of the following soluble adhesion molecules: sVCAM-1, sICAM-1, sL-selectin and sP-selectin (Diacclone Systems, Besançon, France), according to the manufacturer's instructions. These adhesion molecules, which are expressed on the vascular endothelium, platelets and leukocytes, can also be found in their soluble forms in plasma, reflecting endothelial, platelet, or leukocyte activation [26].

## 2.7. Oxidative stress and nitric oxide (NO) measurements

Reduced RBC-membrane protein thiols were measured after reaction with 5,5'-dithiobis(2-nitrobenzoate) (DTNB) [24]. The standards were done with glutathione. RBC membranes were prepared by hypotonic lysis in a Tris/HCl buffer containing phenylmethylsulphonyl fluoride. After incubation with Ellman's reagent (DTNB), reduced protein thiol levels were determined by measuring absorbance at 412 nm against appropriate blanks.

Lipid peroxidation of RBCs was estimated by the measurement of thiobarbituric acid-reactive substance (TBARS), as described by Stocks and Dormandy [28], using a 1,1,3,3-tetraethoxypropane as a standard. RBCs were washed and diluted ten times in phosphate-buffered saline before being lysed in trichloroacetic acid. After incubation and boiling with thiobarbituric acid, the TBARS level was determined by measuring absorbance at 532 nm after reaction with thiobarbituric acid in RBC extracts.

Nitric oxide (NO) production was estimated by measuring plasma concentrations of nitrate/nitrite (after the conversion of nitrate to nitrite utilizing nitrate reductase) with a colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA), to assess the vasodilatory reserve potential [8].

## 2.8. Hemorheological measurements

Hemorheological parameters were measured within 3 h after blood collection in EDTA tubes. Blood viscosity ( $\eta_b$ ) was measured with a cone/plate viscometer (Brookfield DVII+, with CPE40 spindle) at high shear rate (225 s<sup>-1</sup>) at 25°C. The RBC elongation indexes (EI) were determined at 37°C and at seven shear stresses ranging from 0.95 to 30 Pa by laser diffraction analysis, using the Laser-assisted Optical

147 Rotational Cell Analyzer (LORCA, RR Mechatronics, Hoorn, The Netherlands). The system calculates  
148 an average RBC EI for each shear stress. The higher this index is, the more deformable the RBC are [13].  
149 The EI values were plotted as a function of shear stress and the shear stress required for half-maximal  
150 deformation ( $SS_{1/2}$ ) and the predicted maximal EI ( $EI_{max}$ ) were determined by fitting Lineweaver-Burk  
151 curve [4]. Increased  $SS_{1/2}$  values indicate decreased RBC deformability.

152 RBC aggregation was determined at 37°C via syllectometry (i.e., laser backscatter versus time) using  
153 the LORCA, after adjustment of Hct to 40% [3]. The system give various indices of RBC aggregation such  
154 as: 1) RBC aggregation index (AI); 2) minimal shear rate to prevent aggregation ( $\gamma_{thr}$ ) [12]; 3) amplitude  
155 of signal change during aggregation (AMP); 4) half-time for aggregation ( $t_{1/2}$ ).

## 156 2.9. Correction for plasma volume shift

157 Changes in plasma volume were calculated from the measurements of Hct according to the method  
158 described by van Beaumont [34]. The hematological parameters and some coagulation parameters were  
159 corrected accordingly, when necessary [30].

## 160 2.10. Statistical analysis

161 The results are presented as mean  $\pm$  SD. Anthropometric characteristics, exercise responses to the first  
162 progressive and maximal exercise test, and the workload during the submaximal experimental trial were  
163 compared between the two groups using a Student *t* test. The data related to blood rheology, hematology,  
164 adhesion, oxidative stress and nitrite obtained at rest, the end of exercise, and 2 and 24 h of recovery  
165 ( $T_{rec}$  and  $T_{24}$ ) were compared between the two groups using a two-way analysis of variance (ANOVA)  
166 with repeated measures. The significance level was defined as  $p < 0.05$ . Analyses were conducted using  
167 Statistica (v. 5.5, Statsoft, Tulsa, OK, USA).

## 168 3. Results

### 169 3.1. Subject characteristics and exercise responses

170 The anthropometric (age, height and weight) data were not different between groups (SCT group:  
171  $21.4 \pm 2.4$  yr;  $164.8$  cm  $\pm 8.2$ ;  $65.9$  kg  $\pm 8.1$ ; CONT group:  $21.4$  yr  $\pm 1.1$ ;  $172.7$  cm  $\pm 7.1$ ;  $68.9$  kg  $\pm$   
172  $10.5$ ). The maximal exercise test results ( $HR_{max}$ ,  $\dot{V}O_{2max}$ , and MAP) and the first ventilatory threshold  
173 did not differ between groups, nor did the workload used during the experimental trial (Table 1).

### 174 3.2. Hematological parameters

175 As shown in Table 2, the leukocyte count was increased in the two groups at the end of exercise and  
176 remained higher after 2 h of recovery, before returning to baseline at  $T_{24}$ . Both lymphocyte and monocyte  
177 counts were increased at  $T_{Ex}$  in the two groups but returned to baseline level at  $T_{Rec}$ , while the neutrophil  
178 count was not changed after exercise and was increased over resting values at  $T_{Rec}$ . A significant difference  
179 between the two groups was observed at  $T_{24}$  for the lymphocyte counts, with the SCT group presenting  
180 lower values than the CONT group. Platelet and RBC counts, hemoglobin concentration, percentage of  
181 reticulocytes, MCV and MCHC were not different between the two groups and did not exhibit any change

Table 1  
Subjects' responses to maximal exercise and submaximal workload

	HR <sub>max</sub> (beats/min)	VO <sub>2</sub> max (L/min)	MAP (W)	VT1 (%)	2nd trial workload (W)
SCT group	182 ± 10	3.01 ± 0.62	242 ± 52	55.9 ± 14.7	148 ± 30
CONT group	188 ± 6	3.18 ± 0.52	267 ± 53	54.2 ± 9.3	161 ± 31

Values are mean ± SD. HR<sub>max</sub>, maximal heart rate recorded during the maximal exercise; VO<sub>2</sub>max, maximal oxygen consumption; MAP, maximal aerobic power; VT1, first ventilatory threshold, and submaximal workload.

Table 2  
Hematological measurements at rest, after 15 min of intense exercise, and after 2 and 24 h of recovery

		T <sub>R</sub>	T <sub>Ex</sub>	T <sub>Rec</sub>	T <sub>24</sub>
Leukocytes (10 <sup>9</sup> /L)	SCT group	5.33 ± 1.85	7.82 ± 1.91†	6.56 ± 2.38†	5.4 ± 1.71
	CONT group	5.12 ± 1.06	7.33 ± 0.99†	7.47 ± 2.33†	6.05 ± 1.34
Neutrophils (10 <sup>9</sup> /L)	SCT group	2.80 ± 1.56	3.48 ± 1.93	4.47 ± 2.13†	3.29 ± 1.75
	CONT group	2.70 ± 0.93	3.35 ± 0.98	5.25 ± 2.31†	3.13 ± 0.84
Lymphocytes (10 <sup>9</sup> /L)	SCT group	1.76 ± 0.45	3.25 ± 0.71†	1.59 ± 0.36	1.67 ± 0.42*
	CONT group	1.73 ± 0.28	3.05 ± 0.57†	1.50 ± 0.38	2.16 ± 0.65
Monocytes (10 <sup>9</sup> /L)	SCT group	0.49 ± 0.22	0.79 ± 0.23†	0.56 ± 0.22	0.53 ± 0.17
	CONT group	0.44 ± 0.10	0.67 ± 0.15†	0.51 ± 0.13	0.52 ± 0.18
Hct (%)	SCT group	42.3 ± 4.1	46.1 ± 4.1†	42.6 ± 3.4	43.3 ± 3.1
	CONT group	40.9 ± 3.1	45.1 ± 3.8†	42.8 ± 3.0	42.2 ± 3.8
Fibrinogen (g/L)	SCT group	2.98 ± 0.49	3.09 ± 0.80	3.05 ± 0.54	3.15 ± 0.55
	CONT group	2.68 ± 0.40	2.40 ± 0.90	2.78 ± 0.78	2.96 ± 0.76
vWF antigen (%NP)	SCT group	110.9 ± 38.7	134.2 ± 39.5†	133.7 ± 40.3†	111.7 ± 28.0
	CONT group	93.4 ± 42.7	118.1 ± 47.7†	122.2 ± 49.1†	118.6 ± 56.1

Values are mean ± SD. Hct, Hematocrit; vWF, von Willebrand factor expression.

\*Different from CONT group ( $P < 0.05$ ). † Different from T<sub>R</sub> in the two groups ( $p < 0.05$ ).

related to exercise (data not shown). As indicated in Table 2, Hct was increased at T<sub>Ex</sub> in the two groups and returned to baseline during the recovery (T<sub>Rec</sub> and T<sub>24</sub>).

As shown in Table 2, plasma fibrinogen concentration was not changed by exercise and remained unchanged during the recovery in both groups. The vWF antigen was increased above resting levels at T<sub>Ex</sub> in the two groups and remained higher than baseline levels at T<sub>Rec</sub> before returning to baseline at T<sub>24</sub>.

### 3.3. Adhesion molecule measurements

As shown in Fig. 1A, sL-selectin was increased above resting level at T<sub>Rec</sub> and T<sub>24</sub> in the two groups. However, the percentage of increase at T<sub>Rec</sub> was more than three-fold higher in the SCT group than in the CONT group (+23% and +7%, respectively) as compared to T<sub>R</sub> values. The concentration of sP-selectin was greater in the SCT group compared with the CONT group at all times (i.e., T<sub>R</sub>, T<sub>Ex</sub>, T<sub>Rec</sub> and T<sub>24</sub>;

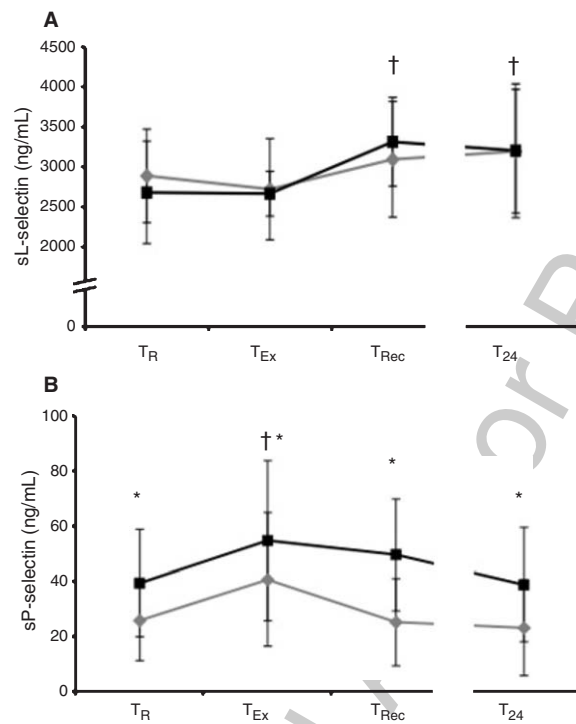


Fig. 1. Time course of plasma concentration of adhesion molecules in soluble form in the SCT group (black line) and the CONT group (gray line). A: sL-selectin, B: sP-selectin. \* Different from CONT group ( $p < 0.05$ ). † Different from T<sub>R</sub> in the two groups ( $p < 0.05$ ).

Fig. 1B). A significant increase in sP-selectin above baseline was observed at T<sub>Ex</sub> in the two groups. The sVCAM-1 and sICAM-1 levels did not differ between the two groups at any time (data not shown).

### 3.4. Oxidative stress and NO measurements

The levels of reduced membrane thiols and TBARS were expressed as percentages of resting values. Neither exercise nor group effects were observed for the reduced membrane thiol kinetics (Fig. 2A). The TBARS were not changed by exercise in the CONT group, but tended to increase above baseline at T<sub>Rec</sub> in the SCT group ( $p = 0.10$ ; Fig. 2B).

Plasma concentration of nitrite did not exhibit any difference between the two groups at T<sub>R</sub> and was not statistically affected by exercise. However, despite the absence of significant change in nitrite at T<sub>24</sub> compared with T<sub>R</sub> in the two groups, the SCT group exhibited slightly but significantly lower values than the CONT group at that time (Fig. 2C).

### 3.5. Hemorheological parameters

As seen in Fig. 3, blood viscosity increased in the two groups during exercise (i.e., T<sub>Ex</sub>), but this increase was greater in the SCT group since the values were significantly higher at T<sub>Ex</sub>, T<sub>Rec</sub> and T<sub>24</sub> than in the CONT group. Moreover,  $\eta_b$  remained higher than baseline at T<sub>Rec</sub> and T<sub>24</sub> in the SCT group only.



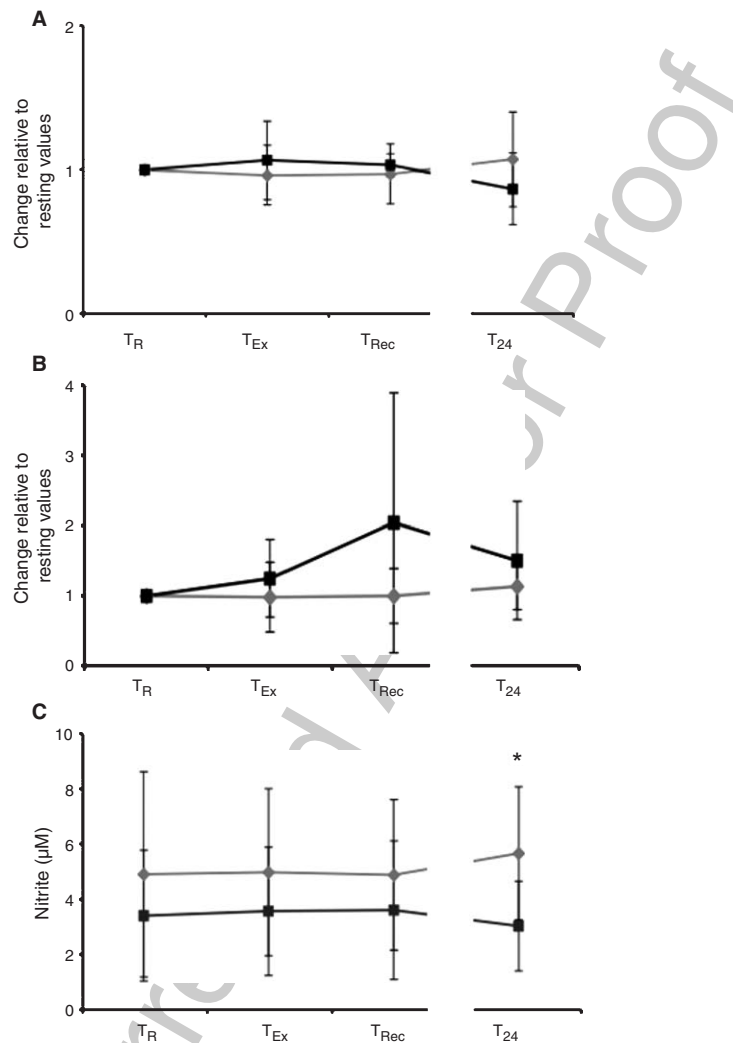


Fig. 2. Kinetics of oxidative stress parameters in the SCT group (black line) and the CONT group (gray line). A: reduced membrane thiols, B: TBARS (thiobarbituric acid-reactive species), C: Time course of plasma concentration in nitrite. \* Different from CONT group ( $p < 0.05$ ).

207 Exercise increased AI in both groups but no group effect was observed at any time (Table 3). In contrast,  
 208 the SCT group exhibited a slightly, but significantly higher disaggregation threshold (i.e.,  $\gamma_{thr}$ ) than the  
 209 CONT group (Table 3). Exercise did not significantly change  $\gamma_{thr}$  in either group. EI was not significantly  
 210 changed by exercise at any shear stress (Table 3). Furthermore, at shear stresses ranging from 0.95 to  
 211 5.33 Pa, EI did not exhibit any significant differences between the two groups (Table 3). In line with this  
 212 finding, the  $SS_{1/2}$ , which was about 3–3.5, did not differ between the groups at any measurement time  
 213 (Fig. 4A). In contrast, the SCT group exhibited a slightly but significantly lower RBC deformability than  
 214 the CONT group at all time-points when measurements were performed at higher shear stresses (i.e.,  
 215 9.49, 16.87 and 30 Pa) (Table 3). Indeed,  $EI_{max}$  was always slightly lower in SCT carriers compared with  
 216 the CONT group (Fig. 4B).

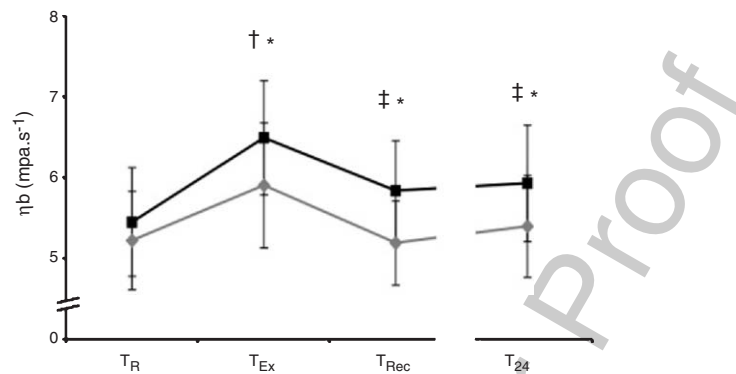


Fig. 3. Time course of  $\eta_b$  measured at a shear rate of  $225 \text{ s}^{-1}$ , in the SCT group (black line) and the CONT group (grey line). \*Different from CONT group ( $p < 0.05$ ). † Different from  $T_R$  in the two groups ( $p < 0.05$ ). ‡ Different from  $T_R$  only in the SCT group.

Table 3

AI,  $\gamma_{thr}$  and EI at seven different shear stresses, at rest, after 15 min of intense exercise, and after 2 and 24 hr of recovery

		$T_R$	$T_{Ex}$	$T_{Rec}$	$T_{24}$
AI	SCT group	$58.12 \pm 10.77$	$62.99 \pm 8.32^\dagger$	$56.18 \pm 10.10$	$60.60 \pm 8.17$
	CONT group	$56.94 \pm 8.41$	$62.57 \pm 7.53^\dagger$	$57.09 \pm 8.55$	$59.40 \pm 7.95$
$\gamma_{thr} \text{ (s}^{-1}\text{)}$	SCT group	$168.6 \pm 47.8^*$	$173.2 \pm 31.5^*$	$170.0 \pm 49.4^*$	$179.8 \pm 57.3^*$
	CONT group	$140.7 \pm 54.6$	$137.3 \pm 41.8$	$134.3 \pm 51.0$	$128.2 \pm 42.7$
EI at 0.95 Pa	SCT group	$0.131 \pm 0.040$	$0.134 \pm 0.027$	$0.217 \pm 0.255$	$0.129 \pm 0.031$
	CONT group	$0.139 \pm 0.025$	$0.131 \pm 0.020$	$0.142 \pm 0.028$	$0.129 \pm 0.020$
EI at 1.69 Pa	SCT group	$0.221 \pm 0.043$	$0.227 \pm 0.029$	$0.230 \pm 0.037$	$0.221 \pm 0.033$
	CONT group	$0.235 \pm 0.027$	$0.228 \pm 0.023$	$0.240 \pm 0.031$	$0.227 \pm 0.021$
EI at 3 Pa	SCT group	$0.317 \pm 0.038$	$0.323 \pm 0.027$	$0.327 \pm 0.034$	$0.317 \pm 0.030$
	CONT group	$0.335 \pm 0.027$	$0.329 \pm 0.025$	$0.343 \pm 0.032$	$0.329 \pm 0.022$
EI at 5.33 Pa	SCT group	$0.409 \pm 0.031$	$0.413 \pm 0.025$	$0.416 \pm 0.029$	$0.407 \pm 0.027$
	CONT group	$0.427 \pm 0.026$	$0.426 \pm 0.025$	$0.437 \pm 0.030$	$0.425 \pm 0.023$
EI at 9.49 Pa	SCT group	$0.485 \pm 0.025^*$	$0.488 \pm 0.023^*$	$0.489 \pm 0.026^*$	$0.484 \pm 0.023^*$
	CONT group	$0.504 \pm 0.022$	$0.501 \pm 0.022$	$0.509 \pm 0.023$	$0.502 \pm 0.019$
EI at 16.87 Pa	SCT group	$0.539 \pm 0.019^*$	$0.538 \pm 0.021^*$	$0.542 \pm 0.022^*$	$0.539 \pm 0.019^*$
	CONT group	$0.555 \pm 0.019$	$0.552 \pm 0.020$	$0.559 \pm 0.017$	$0.555 \pm 0.016$
EI at 30 Pa	SCT group	$0.577 \pm 0.016^*$	$0.578 \pm 0.019^*$	$0.579 \pm 0.021^*$	$0.578 \pm 0.015^*$
	CONT group	$0.590 \pm 0.019$	$0.587 \pm 0.021$	$0.596 \pm 0.015$	$0.590 \pm 0.015$

Values are mean  $\pm$  SD. EI, elongation index; AI, aggregation index, and  $\gamma_{thr}$ , disaggregation threshold.

\*Different from CONT group ( $p < 0.05$ ). † Different from  $T_R$  in the two groups ( $p < 0.05$ ).

#### 217 4. Discussion

218 Several cases of exercise-related sudden death have been reported in response to submaximal efforts  
 219 [17, 18, 37]. This study gave us the opportunity to compare the impact of a submaximal exercise to that of  
 220 a strenuous exercise that we described in previous studies [31, 32]. In the present study, the submaximal

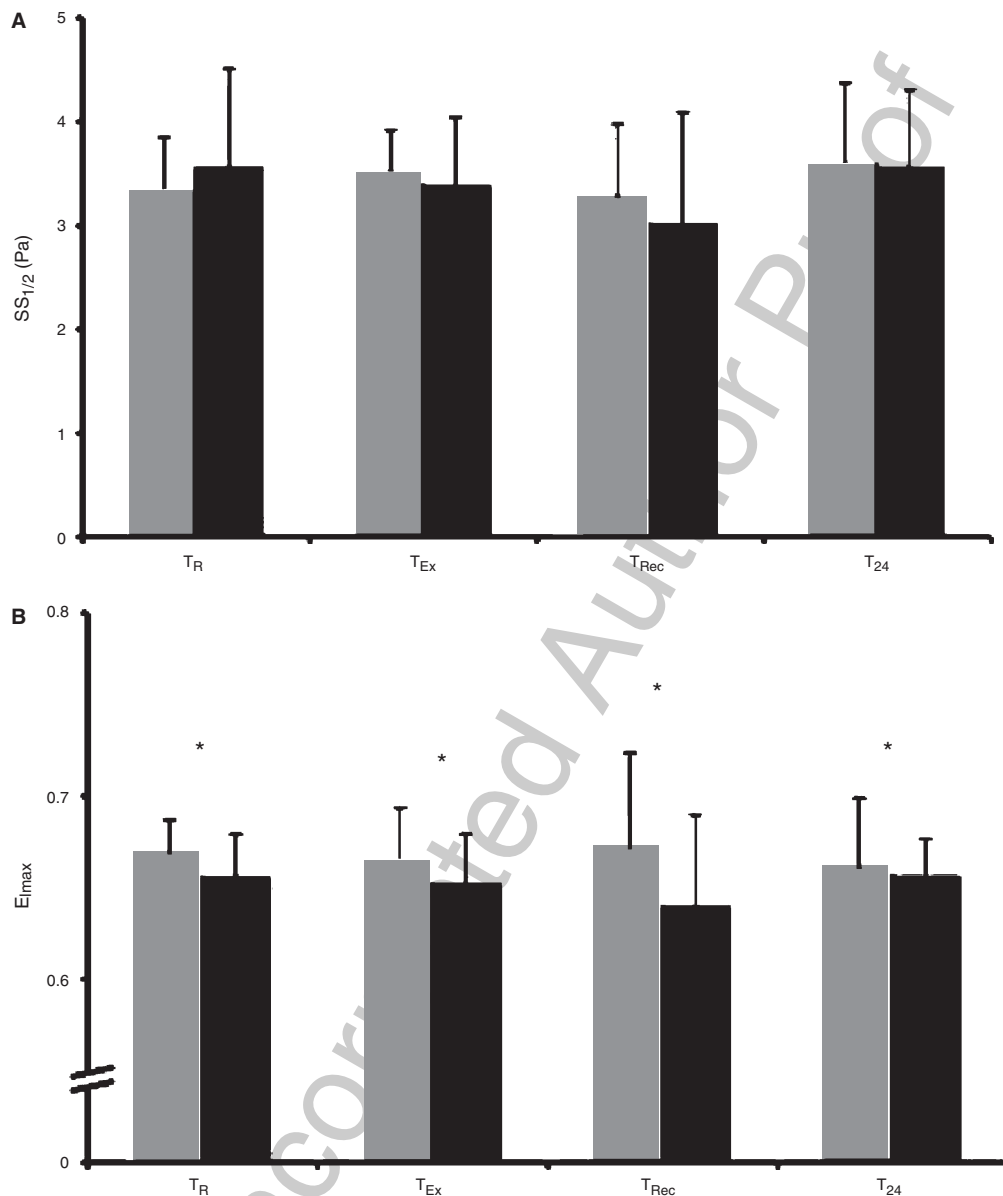


Fig. 4. EI and  $SS_{1/2}$  at  $T_R$ ,  $T_{Ex}$ ,  $T_{Rec}$  and  $T_{24}$  in the SCT group (black bars) and CONT group (gray bars). A:  $SS_{1/2}$ , B:  $E_{I_{max}}$ . \*Different from CONT group ( $p < 0.05$ ).

221 exercise produced a similar increase of blood viscosity in the SCT carriers at the end of exercise and  
 222 during the immediate recovery than did a previous strenuous exercise, but led to a different impact on RBC  
 223 deformability [32]. The higher level of blood viscosity exhibited by the SCT carriers may be partly related  
 224 to the lower RBC deformability found in this group. However, the relationship between the increased blood  
 225 viscosity after the submaximal exercise and RBC deformability remains unclear since RBC deformability  
 226 was unchanged by exercise in the two groups. The previous observations of decreased RBC deformability

227 in SCT carriers, were made using an indirect method based upon high shear rate viscosimetry and the  
228 assessment of an index of RBC rigidity ( $T_k$  index) which is now not supported by the new guidelines  
229 for hemorheological techniques [3]. In the present study, RBC deformability has been determined by  
230 ektacytometry, allowing the measurement of an accurate elongation index. At high shear stress, a slightly  
231 but significantly lower RBC deformability was observed in the SCT group at all time-points (as confirmed  
232 by the lower  $El_{max}$  too) with no changes induced by the submaximal exercise whereas an increased lipid  
233 peroxidation was detected in the SCT group only, and during the immediate recovery. We previously  
234 proposed the involvement of oxidative stress in the greater increase in RBC rigidity above the resting  
235 value in the SCT group than in the CONT group observed during the late recovery of the progressive  
236 and maximal exercise [32]. Therefore, RBC deformability, which was found decreased during the late  
237 recovery of a strenuous exercise but unchanged by the present submaximal exercise, seems to be sensitive  
238 to exercise intensity but the link between oxidative stress and RBC deformability in SCT carriers remains  
239 unclear.

240 Our present results suggest a trend toward greater leukocyte and platelet activation in the SCT carriers  
241 which could have had slight effects on blood viscosity. Decreased polymorphonuclear leucocyte (PMN)  
242 deformability is documented in case of pathologies such as diabetes [22] and chronic renal failure [27],  
243 and it is well established that cellular activation increases PMN rigidity [27, 29].

244 It is usually proposed that RBC aggregation increases with exercise in relation to an increase in plasma  
245 fibrinogen concentration [35]. In the present study, exercise increased RBC aggregation in both groups;  
246 however, we observed no increase in plasma fibrinogen concentration (after correction for plasma volume  
247 change) after exercise, suggesting that other plasmatic or cellular factors were involved. Although of minor  
248 importance, receptor mediated RBC aggregation could have participated to the increased aggregation  
249 during exercise. Since plasma vWF may attach to RBCs via a not yet identified RBC receptor [25],  
250 we hypothesize that the increased plasma vWF concentration with exercise could have contributed to  
251 the increase in AI at  $T_{Ex}$ . Finally, based on recent experimental findings [19], one could suggest an  
252 involvement of the depletion model to explain the increased RBC aggregation during exercise. Although  
253 no difference between the two groups was observed for AI, we observed higher  $\gamma_{thr}$  in the SCT group than  
254 in the CONT group, at every time-point and with no influence of exercise. This indicates that breaking  
255 up the RBC aggregates requires greater forces in SCT carriers [12] than in controls. The mechanisms  
256 underlying this higher  $\gamma_{thr}$  in SCT carriers remain unknown, but the lack of difference between the two  
257 groups for the plasma concentrations of fibrinogen and vWF suggests that cellular factors rather than  
258 plasmatic factors play a role in this hyper-aggregation [23].

259 It is worth noting that the hemorheological alterations observed in the SCT carriers (increased blood  
260 viscosity, increased  $\gamma_{thr}$  and decreased deformability) were relatively small compared with those observed  
261 in sickle cell patients [30] and could be, in fact, subclinical. However, these alterations could have been  
262 more important under conditions leading to RBC dehydration, usually evidenced by parameters such as  
263 increased MCHC and higher RBC distribution width indices, which has not been detected in this study  
264 (data not shown).

265 Despite the small hemorheological differences between the SCT carriers and subjects with normal  
266 Hb, it was important to determine whether the endothelial function was preserved in the SCT carriers to  
267 compensate for these slight alterations. We found no increase in plasma nitrite concentration in response  
268 to exercise in both group and a slightly lower level of plasma nitrite in the SCT carriers at late recovery  
269 ( $T_{24}$ ) suggesting a decreased NO production in SCT carriers during the late recovery of exercise. However,  
270 endothelial function seems to be well preserved in SCT carriers since we did not observe any change in  
271 sICAM-1 or sVCAM-1 from baseline levels during exercise or recovery.

In summary, this study confirmed that SCT carriers have less deformable RBCs than normal subjects and that submaximal exercise, like maximal exercise [32], leads to higher post-exercise blood viscosity, indicating that SCT carriers could be prone to circulatory disturbances during exercise recovery. A new finding is that these alterations did not seem to be related to oxidative stress. Moreover, SCT carriers seem to be characterized by hyper-aggregable RBC that might influence NO production since enhanced RBC aggregation promotes axial migration and results in lower viscosity (and lower shear stress) at vessel wall and decreased NO production [6, 38]. The SCT carriers presented slightly decreased NO production during recovery, which could also be viewed as a vascular risk factor. However, although the production of nitric oxide was slightly reduced in SCT carriers after an exercise bout, endothelial function seems to be well preserved. Moreover, the higher plasma concentration of selectins might reflect a protective mechanism (i.e., a shedding process) against the greater risk for vascular complications that these carriers face (including thrombotic events), especially when exercise is performed under stressful conditions. Fortunately, no exercise-related complications occurred in the present or previous studies focusing on SCT carriers and exercise. It is worth noting that although the hemorheological alterations observed in the SCT carriers are reproducible, they are smaller than those described in sickle cell patients [30]. Their amplitude might depend on the intensity and environmental conditions of the exercise. Prolonged and intense exercise conducted under strenuous environmental conditions, as reported by Hedreville et al. [14], might be associated with red blood cell dehydration and more marked hemorheological alterations, contributing to adverse events.

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