Exercise training blunts oxidative stress in sickle cell trait carriers

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1Center of Research and Innovation on Sports, University Claude Bernard Lyon 1, University of Lyon, Villeurbanne; 2CarMeN, INSERM, University Claude Bernard Lyon 1, Faculty of Medicine Lyon Sud, Oullins; 3Laboratory of Exercise Physiology, University of Savoie and University of Saint-Etienne, Le Bourget du Lac and Saint-Etienne, France; 4Unit of Myology, University Hospital Center of Saint Etienne, France; 5Laboratory of Physiology, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon; 6Laboratoire de Bioénergétique Fondamentale et Appliquée, INSERM, University Joseph Fourier, Grenoble; and 7Unité de Pathologie Moléculaire de l’Hémostologie, Hospices Civils de Lyon, Lyon, France

Chirico EN, Martin C, Faës C, Féasson L, Oyono-Enguéllé S, Aufradet E, Dubouchaud H, Francina A, Canet-Soules E, Thiriet P, Messonnier L, Pialoux V. Exercise training blunts oxidative stress in sickle cell trait carriers. J Appl Physiol 112: 1445–1453, 2012. First published February 9, 2012; doi:10.1152/japplphysiol.01452.2011.— The aim of this study was to analyze the effects of exercise training on oxidative stress in sickle cell trait carriers. Plasma levels of oxidative stress [advanced oxidation protein products (AOPP), protein carbonyl, malondialdehyde (MDA), and nitrotyrosine], antioxidant markers [catalase, glutathione peroxidase (GPX), and superoxide dismutase (SOD)], and nitrite and nitrate (NOx) were assessed at baseline, immediately following a maximal exercise test (Tex), and during recovery (T1h, T2h, T24h) in trained (T: 8 h/wk minimum) and untrained (U: no regular physical activity) sickle cell trait (SCT) carriers or control (CON) subjects (T-SCT, n = 10; U-SCT, n = 8; T-CON, n = 11; and U-CON, n = 11; age: 23.5 ± 2.2 yr). The trained subjects had higher SOD activities (7.6 ± 5.4 vs. 5.2 ± 3.1 U/ml, P = 0.016) and lower levels of AOPP (142 ± 102 vs. 177 ± 102 µM, P = 0.028) and protein carbonyl (82.1 ± 26.0 vs. 107.3 ± 30.6 nm/ml, P = 0.010) than the untrained subjects in response to exercise. In response to exercise, U-SCT had a higher level of AOPP (224 ± 130 vs. 174 ± 121 µM, P = 0.012), nitrotyrosine (127 ± 29.1 vs. 70.6 ± 46.6 nM, P = 0.003), and protein carbonyl (114 ± 34.0 vs. 86.9 ± 26.8 nm/ml, P = 0.006) compared with T-SCT. T-SCT had a higher SOD activity (8.50 ± 7.2 vs. 4.30 ± 2.5 U/ml, P = 0.002) and NOx (28.8 ± 11.4 vs. 14.6 ± 7.0 µmol·l⁻¹·min⁻¹, P = 0.003) in response to exercise than U-SCT. Our data indicate that the overall oxidative stress and nitric oxide response is improved in exercise-trained SCT carriers compared with their untrained counterparts. These results suggest that physical activity could be a viable method of controlling the oxidative stress. This could have a beneficial impact because of its involvement in endothelial dysfunction and subsequent vascular impairment in hemoglobin S carriers.

antioxidants; physical activity; hemoglobin

SICKLE CELL DISEASE (SCD) is a hemoglobinopathy resulting from a single mutation in the β-globin chain gene, inducing the substitution of valine for glutamic acid at the sixth amino acid position. This mutation leads to the production of abnormal hemoglobin (HbS). The pathogenesis of SCD occurs due to the polymerization of deoxygenated HbS, eventually leading to the rigidity and sickling of red blood cells (RBC). The most deleterious pathophysiological effects of sickling include endothelial dysfunction, inflammation, and vaso-occlusion (65). All these effects can be traced back to an increase in oxidative stress, defined as a damaging imbalance between the production of oxidants and antioxidants (63, 66). Endothelial dysfunction, which can cause increased adhesion in vessels leading to vaso-occlusion, hypoxia, and hemolysis, is notably due to the impairment of nitric oxide bioavailability (1, 35). Vaso-occlusion and hypoxia can generate superoxide, whereas hemolysis can inhibit NO production while generating more reactive oxygen species (66).

Subjects who present both normal [hemoglobin A (HbA)] and sickled hemoglobin (HbS) are identified as sickle cell trait (SCT) carriers. SCT is usually considered to be a benign and asymptomatic condition (20). However, several authors suggest that SCT should be reclassified as a disease state (9, 10, 32). In fact, SCT has been linked as a cofactor for morbidity and mortality (32, 60, 69) due to complications at rest (69) and during exercise (30, 31, 34) particularly in hypoxic conditions (31). An increasing number of studies have reported exercise-related sudden deaths in SCT carriers (14, 20, 31, 34). The high incidence of exercise-related deaths in SCT could be a result of RBC abnormalities, such as decreased RBC deformability (32, 68) and endothelial damage (69), associated with an increase in oxidative stress (13, 23). This hypothesis is supported by the fact that (1) RBC sickling may increase during exercise in SCT carriers (6), (2) sickle erythrocytes overproduce reactive oxygen species (ROS) (13, 23), (3) SCT carriers increase RBC oxidative stress during exercise (12), and (4) ROS induce a cascade of events including endothelial dysfunction and adhesion potentially leading to vascular occlusion (1). Taken together, these results lead to the hypothesis that oxidative stress could be involved in exercise-related complications through the vascular dysfunction mechanism seen in SCT.

In various situations, exercise training has been shown to decrease oxidative stress through an upregulation in the antioxidant system, thereby halting the overproduction of oxidants (28, 53–55). In turn, this may improve cardiovascular function by reducing endothelial dysfunction, inflammation, and adhesion (24, 35). A recent paper by Aufradet al. (3) demonstrated that the increased endothelial activation commonly occurring in SCT (47, 48) was attenuated in trained SCT carriers compared with the untrained carriers. This effect on adhesion molecules, which are regulated by nitric oxide and stimulated by ROS (33), suggests that a training effect could be

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under the control of oxidative stress. However, to our knowledge there are no studies dealing with the effects of regular exercise training on oxidative stress and nitric oxide metabolism in SCT carriers.

The aim of the present study is to test the hypothesis that regular training improves NO metabolism and decreases oxidative stress, adhesion, and endothelial dysfunction in SCT subjects.

METHODS

Ethical Approval

The protocol was approved by the local ethics committee of Cameroon and was in accordance with the guidelines set by the Declaration of Helsinki. All of the subjects were volunteers and gave their written informed consent before participating in the study.

Subjects

Eighteen SCT carriers (34.5 ± 0.8% HbS) and 22 subjects with normal hemoglobin (CON) participated in the study. All subjects were male students attending the University of Yaoundé II (Soo, Cameroon). SCT and CON groups were divided into two subgroups on the basis of their fitness level. The 11 CON and 8 SCT who reported no regular physical activity for the two previous years were assigned to untrained (U) subgroups (U-CON and U-SCT). The 11 CON and 10 SCT who practiced soccer on a regular basis (>8 h/wk minimum for several years) were assigned to trained (T) subgroups (T-CON and T-SCT). The group categorization of trained and untrained was confirmed by fitness level measured by relative maximal aerobic power as described in the results. Subjects completed a medical examination that included height and weight measurements, as well as a venous blood test to detect SCT and HIV. Exclusion criteria included the presence of a known chronic disease (hypertension, HIV, stroke, or recent malaria episode). No previous sickle cell crisis or other incident relating to hemoglobinopathy was reported in any subject.

Exercise Protocol

All the experiments took place at General Hospital of Yaoundé (Cameroon). The subjects were asked to avoid any strenuous exercise the day before the test. All meals before and after the exercise test were provided at the hospital and water was available ad libitum to ensure proper nutrition and hydration. An incremental maximal exercise test was performed on a cycle ergometer (Monark, 818E, Stockholm, Sweden). The test began with a 5-min warm up at 30 W, followed by a work rate of 70 W that was continuously increased by 35 W every 3 min until volitional exhaustion was reached. Heart rate (HR) was collected throughout the exercise using a chest belt monitor (Polar Electro, Kempele, Finland). Maximal HR (HRmax, beats/min) was considered the highest recorded heart rate during the test. Maximal aerobic power (MAP; W) was assessed by linear interpolation from the HR vs. work rate curve. Blood samples were drawn from a catheterized antecubital vein on the nondominant arm and were collected at baseline (Base), immediately at the end of the exercise test (T ex), and after 1 h, 2 h, and 24 h of recovery (T 1h, T 2h, T 24h), respectively in EDTA tubes. The samples were used to measure levels of oxidative stress [advanced oxidation protein products (AOPP), protein carbonyl, malondialdehyde (MDA), and nitrotyrosine], antioxidant [catalase, glutathione peroxidase (GPX), and superoxide dismutase (SOD)], and NO metabolism (NOx) markers, and adhesion molecules (P-selectin and E-selectin).

SCT Confirmation, α-Thalassemia, and Hematological Parameters

To test for SCT, blood samples were collected in EDTA tubes at rest and the various Hb were isolated and quantified by high-performance liquid chromatography (HPLC) (Variant I, Beta Thal Short Program: Bio-Rad Laboratories, Hercules, CA). Positive test results for SCT were determined by the presence of HbS, but only if <50% of total Hb. To test for the coexistence of α-thalassemia, the technique described by Chong et al. (8) was used. The only type of α-thalassemia found in some SCT carriers was the heterozygous form marked by a deletion of 3.7 kb of DNA, containing one of the two linked α-globin genes (αa/α-7). No other genetic Hb abnormality was found in this population. Blood for the hematological measurements was collected at rest in EDTA tubes and was analyzed using a hematology analyzer (Abbott Cell Dyn 1800 hematology analyzer, Block Scientific, NY).

Oxidative Stress and Antioxidant Assessment

The blood samples collected at baseline, T ex, T 1h, T 2h, and T 24h were centrifuged, and the aliquoted plasma was stored at −80°C until analysis. All samples were assessed within the same time period.

Plasma AOPP. AOPP were determined in blood plasma using the semi-automated method developed by Witko-Sarsat et al. (70), as previously described (55). AOPP were measured by spectrometry on a microplate reader (TECAN Infinite M200, Mannedorf, Switzerland) and were calibrated with a chloramine-T solution that absorbs at 340 nm in the presence of potassium iodide. The absorbance of the reaction was immediately read at 340 nm on the microplate reader against a blank containing 200 μl of PBS. AOPP activity was expressed as micromoles per liter of chloramines-T equivalents.

Protein carbonyl. Measurements of protein carbonyls can be used as an index of oxidative injury. Protein carbonyls were measured by spectrophotometry at 380 nm using 2,4-dinitrophenylhydrazine according to a method of Levine et al. (43).

Catalase. Catalase activity in the plasma was determined by the method of Johansson and Borg (29), using hydrogen peroxide (H2O2) as a substrate and formaldehyde as a standard. Catalase activity was determined by the formation rate of formaldehyde induced by the reaction of methanol and H2O2 using catalase as enzyme.

GPX. GPX in the plasma was determined by the modified method of Paglia and Valentine (52), using H2O2 as a substrate. GPX was determined by the rate of oxidation of NADPH to NADP+ after addition of glutathione reductase (GR), reduced glutathione (GSH), and NADPH.

MDA. Although MDA assay shows methodological limitations (42), it is the most common lipid peroxidation marker and it is still widely used as marker of oxidative stress. Concentrations of plasma MDA were determined as thiobarbituric reactive substances by a modified method of Ohkawa et al. (51), as previously described (53).

NOx. The end-products of endothelin nitric oxide and NOx were measured in the plasma using reagents purchased from Sigma-Aldrich based on methods previously described (45). The sum of nitrite and nitrate in the plasma (NOx) is considered an index of nitric oxide production (23).

Nitrotyrosine. Concentrations of plasma nitrotyrosine, as end product of protein nitration by ONOO−, were measured as previously described (19). Nitrotyrosine was measured using reagents purchased from Sigma-Aldrich.

SOD. The quantitative determination of the SOD activity was performed using the Beauchamps and Fridovich’s method (5), slightly modified by Oberley and Spitz (50). SOD activity was determined by the degree of inhibition of the reaction between superoxide radicals, produced by a hypoxanthine-xanthine oxidase system, and nitroblue tetrazolium.

Adhesion molecule assessment. sP-selectin and sE-selectin were assessed by ELISA according to the manufacturer’s instructions (Diaclone Systems, Besançon, France).
**Statistical Methods**

The results are presented as mean ± SD. Anthropometric and hematologic characteristics were compared using a two-way ANOVA with Fisher least significant difference (LSD) post hoc. The data related to oxidative stress markers and antioxidants were compared between groups using a two-way repeated-measures ANOVA with Fisher LSD post hoc. Pearson’s coefficient correlations were used to determine the associations between markers of oxidative stress and NOx, nitrotyrosine and adhesion markers, respectively. Statistical significance was determined by a P value of <0.05. Analyses were conducted using Statistica (version 8.0, Statsoft, Tulsa, OK).

**RESULTS**

**Anthropometric and Hematologic Characteristics**

Data for anthropometric, hematologic, and training measurements are detailed in Table 1. Compared with the untrained subjects, the exercise trained group had a significantly higher absolute and relative MAP (169 ± 50 vs. 214 ± 34 W, P = 0.002 and 2.78 ± 0.4 vs. 3.17 ± 0.5 W/kg, P = 0.006, for U and T, respectively). Furthermore, there is no differences in absolute or relative MAP among the U and T subgroups, i.e., between U-SCT and U-CON (nonsignificant: NS) and between T-SCT and T-CON (NS). There were no significant differences in %HbS between U-SCT and T-SCT. Whereas no significant differences appeared in age, RBC count, or maximal heart rate among the four groups, U-SCT was taller and U-CON was lighter than the other three groups (P < 0.05). T-SCT subjects had a significantly higher platelet count than U-SCT and U-CON (see Table 1).

**Oxidative Stress Markers at Baseline and After Maximal Exercise**

**AOPP.** AOPP concentrations were not different among the four groups at baseline (NS) but were significantly higher at Tex than at baseline when the four groups were pooled (P < 0.001; Table 2). Furthermore, AOPP concentrations were significantly higher in U-SCT than in the three other groups (training × hemoglobin crossed effect, P < 0.05).

**MDA.** Baseline, trained subjects had higher levels of MDA than untrained subjects (P = 0.014), whereas there were no differences between SCT carriers and CON subjects. MDA concentrations were significantly increased at Tex, T1h, and T2h compared with baseline in U-SCT subjects, whereas they decreased at T1h, T2h, and T24h in trained subjects (Table 2). The percentage increase from baseline was significantly higher in U-SCT than the three other groups at Tex, T1h, T2h, and T24h (Fig. 1).

**Protein carbonyl.** There was no difference in protein carbonyl content among groups at baseline (NS). Protein carbonyl was significantly higher in all four groups at Tex compared with baseline (U-CON: +117%, P = 0.0002; U-SCT: +124%, P < 0.0001; T-CON: +77%, P < 0.0001; T-SCT: +71%, P = 0.0005). U-SCT protein carbonyl concentrations were significantly higher than in the other three groups at Tex (P = 0.003 vs. T-CON; P = 0.036 vs. U-CON; and P = 0.005 vs. T-SCT). Protein carbonyl levels at T1h, T2h, and T24h were not significantly different from baseline values regardless of the group (Table 2).

**Nitrotyrosine.** Nitrotyrosine levels were not different at baseline among the four groups (NS). An overall training effect (ANOVA trained vs. untrained independently to time point) was observed with higher nitrotyrosine concentrations in untrained subjects compared with their trained counterparts (P = 0.01). U-SCT was the only group with a significant increase in nitrotyrosine at Tex compared with base (P = 0.001). Thus U-SCT had higher nitrotyrosine concentrations at Tex than the other three groups (P = 0.048 vs. U-CON; P < 0.0001 vs. T-CON; and P = 0.003 vs. T-SCT). At T1h, U-CON, U-SCT, and T-CON exhibited higher nitrotyrosine concentrations than at baseline (Table 2).

**Antioxidant Markers at Baseline and After Maximal Exercise**

**SOD.** No significant inter-group difference was observed in baseline SOD activity. Compared with baseline, a significant increase of SOD activity was observed at Tex (P < 0.0001) and T1h (P = 0.002) in trained subjects, whereas no significant variations were observed in their untrained counterparts (Table 3). Furthermore, SOD activity was significantly higher in the trained subjects than in the untrained ones at Tex (P = 0.015; Fig. 2).

**Catalase.** A training effect was observed at baseline with significantly higher catalase activities in trained subjects than in their untrained counterparts (P < 0.001). Trained subjects expressed lower activities of catalase at Tex (P < 0.001), T2h (P < 0.001) and T24h (P < 0.001) than at baseline (Table 3).

**GPX.** No significant inter-group differences were observed at baseline for GPX activity. Regardless of the training or

Table 1. Anthropometric, hematologic, and training measurements of the study population

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>SCT</th>
<th>Untrained</th>
<th>Con</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>%HbS</td>
<td>N/A</td>
<td>36.2 ± 3.1</td>
<td>N/A</td>
<td>33.2 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>α-Thal</td>
<td>N/A</td>
<td>3/8</td>
<td>N/A</td>
<td>7/10</td>
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<tr>
<td>Age, yr</td>
<td>22.7 ± 1.8</td>
<td>23.5 ± 3.0</td>
<td>24.6 ± 1.4</td>
<td>23.10 ± 2.3</td>
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<tr>
<td>Height, cm</td>
<td>169.2 ± 3.8*</td>
<td>178.0 ± 4.4</td>
<td>173.7 ± 5.5*</td>
<td>172.1 ± 3.1*</td>
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<tr>
<td>Weight, kg</td>
<td>60.1 ± 5.7</td>
<td>69.5 ± 5.5†</td>
<td>69.2 ± 4.8†</td>
<td>65.6 ± 6.1†</td>
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<tr>
<td>RBC</td>
<td>5.2 ± 0.7</td>
<td>5.3 ± 0.5</td>
<td>5.1 ± 0.1</td>
<td>5.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>164.1 ± 81.0</td>
<td>172.7 ± 82.5</td>
<td>206.6 ± 95.2</td>
<td>266.9 ± 54.7</td>
<td></td>
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<tr>
<td>MAP, W</td>
<td>154.1 ± 16.7</td>
<td>190.4 ± 11.5</td>
<td>214.6 ± 11.3</td>
<td>212.5 ± 10.4</td>
<td></td>
</tr>
<tr>
<td>Max HR, beats/min</td>
<td>185 ± 5</td>
<td>183 ± 4</td>
<td>174 ± 3</td>
<td>189 ± 4</td>
<td></td>
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<tr>
<td>Relative MAP, W/kg</td>
<td>2.81 ± 0.36‡</td>
<td>2.73 ± 0.35‡</td>
<td>3.11 ± 0.53</td>
<td>3.24 ± 0.43</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as means ± SD. CON, healthy subjects; SCT, carriers of sickle cell trait; MAP, maximal aerobic power; %HbS, percent of S hemoglobin.

*Significant difference compared with untrained (U)-SCT (P < 0.05); †significant difference compared to U-CON (P < 0.05); ‡significant difference compared to trained subjects (CON and SCT, P < 0.05).
hemoglobin status, GPX was significantly higher at T_{ex}, T_{1h}, and T_{2h} compared with baseline (baseline: 46.3 ± 18.8 vs. T_{ex}: 111.6 ± 31.7, P < 0.0001, vs. T_{1h}: 102.9 ± 21.6 P < 0.0001, and vs. T_{2h}: 77.9 ± 21.6, P < 0.0001; 4 groups pooled). At T_{1h} GPX was significantly higher in the trained subjects than in their untrained counterparts (112.0 ± 14.7 vs. 87.7 ± 9.5, respectively, P = 0.041; Table 3).

**NOx.** No significant inter-group difference was observed in baseline NOx. NOx was significantly higher in trained subjects, whatever their hemoglobin status (i.e., T-SCT or T-CON).

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**Table 2.** Plasma AOPP, MDA, nitrotyrosine, and protein carbonyl at Base, immediately after a maximal T_{ex}, and during T_{1h}, T_{2h}, T_{24h} in U-CON, U-SCT, T-CON, and T-SCT

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
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<th>Trained</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>CON SCT</td>
<td></td>
<td>CON SCT</td>
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<tr>
<td></td>
<td></td>
<td>Time</td>
<td>Effect</td>
<td></td>
</tr>
<tr>
<td>AOPP, μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>61.2 ± 24.7</td>
<td>78.7 ± 53.6</td>
<td>80.9 ± 48.5</td>
<td>64.4 ± 16.5</td>
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<td>T_{ex}</td>
<td>127.7 ± 49.6</td>
<td>214.0 ± 136.9</td>
<td>110.4 ± 66.9</td>
<td>123.7 ± 65.4</td>
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<td>T_{1h}</td>
<td>73.4 ± 59.3</td>
<td>125.8 ± 81.6</td>
<td>76.9 ± 54.5</td>
<td>57.4 ± 49.4</td>
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<tr>
<td>T_{2h}</td>
<td>99.5 ± 87.6</td>
<td>136.5 ± 87.3</td>
<td>69.9 ± 54.1</td>
<td>88.3 ± 64.1</td>
</tr>
<tr>
<td>T_{24h}</td>
<td>86.0 ± 30.7</td>
<td>124.2 ± 96.6</td>
<td>131.3 ± 75.5</td>
<td>125.6 ± 49.2</td>
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<td>Protein carbonyl, μM</td>
<td></td>
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<tr>
<td>Base</td>
<td>50.5 ± 12.0</td>
<td>49.2 ± 11.4</td>
<td>46.8 ± 14.4</td>
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<td>T_{ex}</td>
<td>89.5 ± 12.0</td>
<td>111.4 ± 36.6</td>
<td>81.6 ± 16.0</td>
<td>84.8 ± 25.0</td>
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<tr>
<td>T_{1h}</td>
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<td>T_{2h}</td>
<td>36.3 ± 6.9</td>
<td>43.5 ± 9.6</td>
<td>52.5 ± 15.6</td>
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<tr>
<td>T_{24h}</td>
<td>37.1 ± 7.4</td>
<td>46.7 ± 18.4</td>
<td>46.2 ± 13.8</td>
<td>41.8 ± 10.3</td>
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<tr>
<td>Nitrotyrosine, nM</td>
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<tr>
<td>Base</td>
<td>82.8 ± 28.7</td>
<td>67.6 ± 20.8</td>
<td>65.4 ± 21.3</td>
<td>95.2 ± 29.4</td>
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<td>T_{ex}</td>
<td>94.3 ± 38.2</td>
<td>132.7 ± 27.7</td>
<td>97.1 ± 39.1</td>
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<td>T_{1h}</td>
<td>130.5 ± 22.3</td>
<td>115.6 ± 32.9</td>
<td>104.9 ± 24.4</td>
<td>92.0 ± 27.0</td>
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<td>T_{2h}</td>
<td>102.1 ± 27.5</td>
<td>95.6 ± 43.4</td>
<td>97.3 ± 34.3</td>
<td>80.0 ± 30.1</td>
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<tr>
<td>T_{24h}</td>
<td>76.7 ± 25.1</td>
<td>83.4 ± 37.0</td>
<td>47.0 ± 29.7</td>
<td>52.0 ± 29.8</td>
</tr>
</tbody>
</table>

Values are presented as means ± SD. AOPP, advanced oxidation protein products; MDA, malondialdehyde. *Significant difference compared with BASE; †significant difference compared with the other times; ‡significant difference compared with T_{ex}. §Significant difference compared with T_{1h} and T_{2h}; ‰significant difference compared with the 3 other groups; †significant difference compared with U-SCT (P < 0.05); ‡significant difference compared with untrained subjects (P < 0.05).

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**Fig. 1.** Effect of a maximal exercise test on the plasma malondialdehyde (MDA) in trained sickle cell trait carriers and controls (T-SCT and T-CON) and untrained (U-SCT and U-CON) subjects compared with baseline (Base) at the end of exercise (T_{ex}), and during recovery (T_{1h}, T_{2h}, T_{24h}). Values are presented as means ± SD. §Significant difference from other 3 groups (P < 0.05).
at T_ex compared with baseline (P = 0.037), whereas NOx remained unchanged in untrained subjects. Consequently, trained subjects had significantly higher NOx values than untrained subjects at T_ex (P = 0.001). Moreover, NOx in U-SCT significantly increased at T_2h compared with baseline (P = 0.0005) and was significantly higher than the three other groups (P = 0.017 vs. U-CON; P = 0.002 vs. T-CON, and P = 0.006 vs. T-SCT).

Adhesion Molecules

To better understand the correlations between oxidative stress and adhesion molecules, the adhesion data previously presented by Aufradet et al. (3) are summarized in this paragraph. Briefly, basal plasma concentrations of sP-selectin and sE-selectin were not statistically different among the four groups. Plasma sE-selectin significantly increased in all groups at the end of the exercise compared with baseline levels and returned to baseline value 1 h after the end of exercise (T1h). Although incremental exercise did not statistically modulate sP-selectin concentrations in T subjects, a significant increase in these concentrations was measured in their untrained counterparts between Base and T_ex. These concentrations returned to basal values 1 h after the end of exercise.

Correlations

Significant correlations were observed between markers of oxidative stress, nitric oxide, and markers of adhesion. The correlations on the pooled subjects are presented in Table 4. We also found significant relationships between changes (Base vs. T_ex, T_1h, or T_2h) in oxidative stress and sE- and sP-selectins or NO metabolism (Table 4). Finally, percentage of HbS was negatively correlated with the NOx increase between baseline and T_ex (r = −0.59, P = 0.021), i.e., the more the HbS content, the lower the increase in NOx in response to exercise.

DISCUSSION

The aim of this study was to investigate the impact of regular physical activity on plasma markers of oxidative stress in SCT.
Table 4. Correlations between oxidative stress and adhesion markers (pooled subjects)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Timepoint</th>
<th>Pearson’s Correlation</th>
<th>P Value</th>
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<td></td>
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<td></td>
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<tr>
<td>AOPP</td>
<td>Baseline</td>
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<td>GPX</td>
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<td>Baseline vs. Ex</td>
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Values are presented as Pearson’s correlation coefficients and corresponding P values.

carriers. In support of our hypotheses, the results of the present study demonstrated that regular physical activity 1) blunted the increase in oxidative stress and the decrease in NO metabolism observed in SCT and 2) upregulated the antioxidant enzymes activities (SOD and GPX) in response to exercise in SCT. In addition, we reported a strong association between changes in various oxidative stress markers in response to exercise and the corresponding changes in sP- and sE-selectins. Although no causality can be assumed, these correlations strengthen the hypothesis that ROS may be involved in endothelial adhesion in HbS carriers.

**General Considerations on the Effects of Hemoglobin and Training Status on Hematologic and Training Measurements**

Maximal aerobic power was similar between SCT and control subjects, as supported by previous studies. Conversely, MAP was higher in the trained subjects compared with the untrained subjects. In agreement with several authors (15, 64), we found that trained subjects had a higher platelet count than untrained subjects. The T-SCT subjects had nonsignificantly higher platelet counts than the other three groups. Although an increased platelet count is a risk factor for vaso-occlusive crises in SCD (62), this increase was not significant and is probably due to a training effect rather than a hemoglobin effect.

**General Considerations on the Effects of Maximal Exercise and Training on Oxidative Stress and Antioxidant Markers in Healthy Subjects**

Regarding the results obtained in CON, the present study is in complete agreement with data reported in the literature. First, the overall increase in various markers of oxidative stress observed in response to the maximal exercise test is consistent with the results of previous studies (7, 41). Second, strong evidence indicates that exercise training can have a beneficial effect on oxidative stress (21, 28, 41). As supported by Miyazaki et al. (46), trained subjects have an improved ability to endure the stress of a maximal exercise bout. A lower level of oxidative stress was also evident in the trained subjects of our study. In fact, T-CON subjects had a reduced increase in all markers of oxidative stress in response to the maximal exercise test compared with their untrained counterparts.

It has been reliably shown that individuals partaking in exercise training have higher levels of antioxidant enzymes and exhibit greater protection against exercise-induced oxidative stress (27, 61). The reactive oxygen species produced during exercise training can result in a stimulation of antioxidant defense (39, 57). The exercise-trained subjects in our study produced a significant increase in SOD and GPX, which was accompanied by lower oxidative stress. This decrease in oxidative stress likely occurs due to the improved antioxidant adaptation. Our results are in agreement with several other studies that found an improved antioxidant response relating to exercise training (25, 53).

Our study also shows higher baseline levels of MDA in our trained subjects (16, 21). The high level of resting MDA in trained subjects could be related to the ability of lipid peroxidation products to regulate and modulate cell signaling and gene expression (49). Oxidized lipids can interact with receptors that are known to activate antioxidant enzymes such as catalase and SOD (22) and can improve cellular tolerance against pro-oxidant attacks (49). It is therefore possible that higher levels of lipid peroxidation products, such as MDA, are needed to maintain antioxidant status, such as we saw for catalase and GPX (even if nonsignificant).

**Exercise Training Blunts Oxidative Stress in SCT Carriers**

HbS, which represents ~80% of total hemoglobin in SCD patients, can generate a twofold greater quantity of ROS than HbA (2, 23). In SCT carriers, in whom HbS represents ~40% of total hemoglobin, we found that oxidative stress varied little at baseline from healthy subjects. This is in agreement with other studies that found no difference between SCT carriers and healthy subjects at baseline (3, 12, 67). However, in response to acute exercise, SCT carriers can experience higher oxidative stress (12, 36). Our study reinforces this idea as it shows that U-SCT had a significantly greater increase in MDA and AOPP in response to a maximal exercise bout than the healthy subjects. The increased morbidity or mortality in SCT could be attributed to higher levels of oxidative stress (32).

However, the main finding of the present study is that the well-known benefits of exercise training in healthy subjects are well translated in the SCT subjects. In response to the maximal exercise test, T-SCT subjects 1) exhibited lower AOPP, MDA, nitrotyrosine, and protein carbonyl levels than U-SCT, and 2) responded similarly to the T-CON subjects with regard to the protein carbonyl, MDA, and AOPP. In addition to the fact that these results mimic those already seen in many other disease, such as cardiovascular disease (17), diabetes (56), and menopause (53), they emphasize the beneficial effects of exercise training on oxidative stress in SCT carriers.

**Antioxidant Defense System is Improved in Exercise-Train SCT Carriers**

The improvement in the antioxidants defense system seen in exercise training is due to a ROS-generated stimulation triggering antioxidant enzyme activation (39, 57). The maximal exercise test that normally increases oxidative stress was met by a concomitant increase in antioxidant enzyme defenses, in
all but the U-SCT carriers. Repeated habitual exercise can cause antioxidants to increase in response to the repeated oxidative stress. This has been supported in vitro where the treatment of pro-oxidants stimulated a significant increase in antioxidants (18). Interestingly, this did not occur in U-SCT subjects, as they had a delayed response of SOD and a reversed response of catalase. This supports studies that showed levels of SOD and catalase decrease in proportion to disease severity (13, 59). The response to SOD and catalase could indicate an impaired antioxidant status, meaning a reduced ability to buffer the excess oxidative stress.

The trait by itself can result in an increase in oxidative stress and impairment in NO, and when paired with an acute stress such as a maximal exercise test, this response is augmented, as observed with U-SCT. However, exercise training is able to stimulate antioxidants to respond to the overload of oxidative stress. This improved response to oxidative stress allows SCT carriers to respond similarly to CON subjects, as demonstrated by our study. This indicates that, as far as oxidative stress is concerned, training can override the negative consequence of SCT, rendering these subjects as controls.

**Nitric Oxide**

Nitric oxide, an important mediator of vasodilation, has been shown to be amplified in response to exercise training (21, 40, 41, 44). The present study supports these findings, as the SCT and CON trained subjects significantly increased NOx immediately after the exercise test, whereas the untrained subjects slightly decreased. Our study suggests that exercise training may inhibit NO degradation through an upregulation of antioxidants and a decrease in ROS. We found that the percent change (%Δ) of NOx levels were positively correlated with GPX levels at T1h and were negatively correlated with MDA levels (%Δ) at T1h. Nitrotyrosine, which represents the nitration activity of peroxynitrite (ONOO−) produced through the reaction of NO and superoxide anion (O2·−), tended to increase in the untrained subjects compared with the trained subjects (P = 0.055) and was significantly higher in the U-SCT carriers compared with T-SCT carriers (P = 0.003). This is in accordance with other studies that found a decrease in nitrotyrosine levels in exercise-trained subjects (37, 53). Although our correlations do not suggest causality, these results I) could indicate a reduced O2·− production and 2) suggest an increase in NO bioavailability as a result of reduced oxidative stress (21, 53) in trained SCT carriers than in their untrained counterparts.

The improvement in NOx levels in response to physical activity level is reflected in the SCT subjects as well. T-SCT subjects had a similar improvement in NOx response as the T-CON subjects. Interestingly, the U-SCT had a delayed peak in NOx at T2h, which was associated with an elevation in oxidative stress. These results emphasize the overall impairment in response to an exhaustive exercise bout. An impaired NO bioavailability is associated with an increase in ROS, hemolysis (58), eNOS uncoupling due to hemolysis-induced arginase, and cell adhesion (35). Because NO has potent anti-adhesive properties which downregulate adhesion molecule expression maintaining proper endothelial cell function and vasodilation, a NO impairment can increase cell adhesion (35). This is supported by Aufradet et al. (3), who found a significant increase in VCAM-1 levels in untrained SCT subjects immediately after exercise (U-SCT: 1,738 ± 98 ng/ml vs. T-SCT: 1,248 ± 131 ng/ml; P < .05)—the exact same time point as the decreased NOx response in U-SCT carriers. As ROS markers were positively correlated with sP- and sE-selectins and negatively correlated with antioxidant markers, these data suggest that a NO-induced increase in oxidative stress could exacerbate endothelial and/or platelets activation. However, it should be noted that there were no differences between sP-selectin and sE-selectin in the different subjects. Therefore, the training-induced decrease in oxidative stress and increase in NO bioavailability could be favorable for the health of SCT carriers involved in regular exercise by dampening risk of sickling, morbidity, and mortality.

This study suggests that exercise training can improve the response to oxidative stress in SCT carriers. However, at this time, most studies involving different intensities and durations of exercise have found conflicting results in relation to coagulation activity, RBC deformability, inflammation, and adhesion in SCT [see review by Connes et al. (11)]. However, these events may be compounded by other factors such as heat stress, dehydration, and poor physical conditioning. Baskurt and Meiselman (4), suggested that exercise utilizes the vascular autoregulatory reserve to maintain homeostasis, yet even minor vascular and hemorheologic perturbations in SCT carriers may be augmented in response to exercise.

**Conclusion**

In conclusion, we found that although there is relatively little difference between SCT carriers and healthy subjects at rest, a maximal exercise test can inundate the oxidative stress response in U-SCT subjects. We also found that training can reduce the oxidative stress in response to exercise of SCT carriers. Training improves antioxidant and NO availability that can thereafter regulate ROS production. These effects could result in decreased endothelial activation. This study does not allow us to make definitive conclusions about the direct causality between exercise training and oxidative stress and NO improvements. Further information could be concluded using longitudinal studies which focus on mechanistic pathways and more directly evaluate endothelial dysfunction using methods such as flow mediated dilation.

Finally, we believe that the beneficial effects seen in SCT carriers in this study could translate well in SCD patients as well. Although there are large clinical differences between SCT and SCD, both are overwhelmed by an increase in oxidative stress. Because we have shown that exercise training can decrease oxidative stress and improve antioxidant and nitric oxide responses, we believe that the complications known in SCD, such as vaso-occlusion crisis, that are linked to oxidative stress (26, 38) could potentially be reduced. There-fore, an adapted exercise training program could be a relevant option to control the cardiovascular complications of this pathology.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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