

## Effects of progressive and maximal exercise on plasma levels of adhesion molecules in athletes with sickle cell trait with or without $\alpha$ -thalassemia

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**Monchanin G, Serpero LD, Connes P, Tripette J, Wouassi D, Bezin L, Francina A, Ngongang J, de la Peña M, Massarelli R, Gozal D, Thiriet P, Martin C.** Effects of progressive and maximal exercise on plasma levels of adhesion molecules in athletes with sickle cell trait with or without  $\alpha$ -thalassemia. *J Appl Physiol* 102: 169–173, 2007. First published August 10, 2006; doi:10.1152/jappphysiol.00272.2006.—The aim of the study was to examine the effects of exercise on soluble vascular cell adhesion molecule-1 (sVCAM-1) and intercellular adhesion molecule-1 (sICAM-1) in sickle cell trait (SCT) athletes with or without  $\alpha$ -thalassemia. Six athletes with SCT, seven athletes with both SCT and  $\alpha$ -thalassemia (SCTAT), and seven control athletes (Cont) performed an incremental and maximal test on cycle ergometer. Levels of sICAM-1 and sVCAM-1 were assessed at rest, immediately after the end of exercise, and 1, 2, and 24 h after exercise. Although Cont and SCTAT groups exhibited similar basal plasma levels of inflammatory and adhesion molecules, the SCT group had higher sVCAM-1 basal concentrations. Incremental exercise resulted in a significant increase of sVCAM-1 in all subjects, which remained elevated only in the SCT group during the recovery period. In conclusion, as sVCAM-1 increased with exercise and during the recovery period, our findings support the concept that SCT athletes might be at risk for microcirculatory disturbances and adhesive phenomena developing at rest and several hours after exercise.  $\alpha$ -Thalassemia might be considered protective among exercising SCT subjects.

tumor necrosis factor- $\alpha$ ; vascular cell adhesion molecule-1; hemoglobinopathy; incremental exercise

SICKLE CELL ANEMIA (SCA) is caused by the mutation of hemoglobin (Hb) A into HbS due to the substitution of a single amino acid, valine for glutamic acid. This mutation leads to polymerization and red blood cell (RBC) shape changes during Hb deoxygenation (24). Sickle cell trait (SCT) carriers are characterized by the presence of both HbA and HbS (AS genotype) in their RBCs. Individuals with SCT are usually asymptomatic, although it has recently been demonstrated that cardiovascular risk might be increased in SCT carriers (1, 8, 17). In addition, SCT has been associated with diminished exercise tolerance (9), increased risk of sudden death, and diminished physical performance under stressful physiological conditions (22, 39). Embury (14) reported that the coexistence of SCA with  $\alpha$ -thalassemia caused by the deletion of a part of the gene encoding for the  $\alpha$ -globin chain occurs frequently in

the black population, and such coexistence may in fact be protective and decrease the severity of health-related complications associated with SCA or SCT alone (27, 34, 37).

We recently reported that SCT carriers exhibited impaired blood rheology (high blood viscosity and low RBC deformability) during resting conditions and that such alterations were dampened by the coexistence of  $\alpha$ -thalassemia (32). Since these hemorheological impairments are thought to increase blood flow resistance in microvessels, they could accelerate endothelial dysfunction and vascular injury (40, 41). Soluble adhesion molecules and vascular adhesion phenomena are potential markers of this endothelial activation (5, 23). In SCA patients, a combination of the trapping of nondeformable cells and the interactions between sickle reticulocytes, leukocytes, and endothelial cells delay the transit time of RBCs through the capillaries and contribute to the initiation and progression of vasoocclusive crises (3, 16, 19, 23). The activation of endothelial cells and their interactions with blood cells are strongly mediated by intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (11), which support transmigration of leukocytes and adhesion of reticulocytes to stimulated endothelium (16). Upregulation of these adhesion molecules on the surface of endothelial cells is prominent when they are exposed to proinflammatory molecules such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (29). Elevated circulating levels of plasma adhesion molecules and inflammatory molecules such as TNF- $\alpha$  have been proposed as correlates of underlying inflammation in patients with SCA at steady state (11, 13, 29) or during vasoocclusive crises (23) and in  $\alpha$ - and  $\beta$ -thalassemic patients (5). However, their role in SCT with or without  $\alpha$ -thalassemia remains undefined, particularly in the context of exercise.

In healthy athletes, blood cell-endothelial cell interactions may occur during and several hours after exercise (33). Therefore, intense exercise may lead to increased plasma levels of ICAM-1 (33), VCAM-1, and circulating TNF- $\alpha$  (36). In contrast, moderately intense exercise impacts only slightly on the level of circulating vascular adhesion molecules (20). Considering the increase in adhesion molecules during exercise in healthy subjects and the presence of HbS in SCT subjects, which could potentiate the adhesive phenomena, we hypothe-

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sized that the “adhesive potential” of SCT carriers may be altered in response to exercise. Thus the aim of the present study was to examine the effects of an incremental and maximal exercise on circulating TNF- $\alpha$ , ICAM-1, and VCAM-1 in SCT athletes with or without  $\alpha$ -thalassemia.

## METHODS

**Subjects.** Twenty well-trained young men volunteered to enter this study and gave their written informed consent. Six athletes were SCT carriers (SCT group), seven had both SCT and  $\alpha$ -thalassemia (SCTAT), and seven athletes with normal Hb comprised the control group (Cont). All athletes were competitive at the national level and were engaged in regular physical training in endurance sports for several years. Athletes were recruited at the National Institute of Youth and Sport in Yaounde (Cameroon) after a complete medical examination including a venous blood test to detect sickle cell trait and  $\alpha$ -thalassemia (see below). Exclusion criteria included the presence of any known chronic disorder or a history of hypertension, stroke, or malaria. No athlete reported a previous sickle crisis or incident in the competitive career.

**Study design.** The protocol was approved by the National Ethics Committee of Cameroon (registration no. FWA IRB00001954) and was in accordance with the guidelines set by the Declaration of Helsinki. On the experimental day, all athletes performed an incremental maximal test exercise until they reached maximal power ( $P_{\text{peak}}$ ). Blood samples were drawn from the finger and from the antecubital vein of the nondominant arm at rest and after exercise tests to measure levels of blood lactate and the soluble adhesion molecules sVCAM-1 and sICAM-1, as well as TNF- $\alpha$  concentrations. Subjects were asked to avoid strenuous exercise and to refrain from consuming caffeine and alcohol 24 h before testing. The laboratory was air conditioned (room temperature 25–27°C, hygrometry 55%).

**Exercise test.** After 5-min warm-up at 30 W, all athletes exercised an incremental test on a mechanically braked ergometer (Ergomeca, Toulon, France). The work rate was increased every minute by 30-W steps until exhaustion and therefore allowed assessment of  $P_{\text{peak}}$  (W). Pedaling rate was set at 70 rpm throughout the test. Heart rate (HR) was continuously monitored using a polar interface.  $P_{\text{peak}}$  was assumed when two of the three following criteria were met: 1) maximal HR corresponding to the age-predicted maximal HR [(220 – age in yr)  $\pm$  10%], 2) inability to maintain the pedaling rate above 60 rpm despite verbal encouragement, 3) maximal blood lactate > 10 mM.

**SCT and  $\alpha$ -thalassemia detections.** Blood was collected at rest in EDTA tubes. The various Hbs (A1, A2, F, S) were isolated and quantified by an ion-exchange high-performance liquid chromatography (Variant I, Beta Thal Short Program; Bio-Rad Laboratories, Hercules, CA). Positive test results for SCT were determined by the presence of HbS but with <50% of total Hb. To study the presence of  $\alpha$ -thalassemia, we used the technique described by Chong et al. (7). The only form of  $\alpha$ -thalassemia found in the SCTAT group was the heterozygous one marked by a deletion of 3.7 kb of DNA, containing one of the two linked  $\alpha$ -globin genes ( $\alpha\alpha/\alpha^{-3.7}$ ).

**Blood lactate assessment.** Fingertip arterialized blood microsamples were taken for lactate analysis before the warm-up and 3 min after the end of the incremental exercise using lactate analyzer (Lactate Pro LT-1710, Roche Bioelectronics, Basel, Switzerland).

**Blood sampling and assessment of soluble cell adhesion molecules.** Venous blood for adhesion and inflammatory molecules was collected in EDTA tubes at rest ( $T_{\text{rest}}$ ); immediately after completion of exercise protocol ( $T_{\text{ex}}$ ); and 1, 2, and 24 h after the end of exercise ( $T_{1\text{h}}$ ,  $T_{2\text{h}}$  and  $T_{24\text{h}}$ , respectively).

Plasma was separated from the collected blood centrifugation and stored at –80°C. ELISA kits (Diacclone Systems, Besançon, France, for sICAM-1 and sVCAM-1; BioSource International, Camarillo, CA,

for TNF- $\alpha$ ) were used to assay the plasma concentrations for TNF- $\alpha$  and for the soluble adhesion molecules sVCAM-1 and sICAM-1 according to the manufacturer’s instructions. These adhesion molecules are expressed on the vascular endothelium and can also be found in their soluble forms in plasma, where they reflect endothelial cell expression (35).

**Statistics.** All the results are shown as means  $\pm$  SE. Data related to subject characteristics and  $P_{\text{peak}}$  were compared between the three groups using one-way ANOVA procedures. Blood lactate and plasma levels of soluble adhesion molecules and TNF- $\alpha$  were compared between the three groups and at different times using two-way ANOVA with repeated measurements followed by paired comparisons as appropriate. Statistical significance was set at  $\alpha = 0.05$ .

## RESULTS

**Anthropometric characteristics and  $P_{\text{peak}}$ .** As shown in Table 1, anthropometric characteristics were similar among SCT, SCTAT, and Cont groups. No significant differences concerning the  $P_{\text{peak}}$  achieved during the incremental exercise test were observed between the three groups. The percentage of HbS was significantly higher in the SCT group compared with the SCTAT group ( $P < 0.05$ ). The percentage of HbA1 and HbA2 were, respectively, higher and lower in the Cont group compared with the two other groups. HbF was not statistically different between the three groups.

**Blood and plasma analysis at rest and after incremental exercise.** Concentrations of sICAM-1 and TNF- $\alpha$  were not statistically different between the three groups at any time during the incremental exercise test (Table 2). However, sVCAM-1 was significantly higher at  $T_{\text{rest}}$ ,  $T_{1\text{h}}$ , and  $T_{2\text{h}}$  in the SCT group than in the two other ones (Fig. 1).

Plasma sVCAM-1 concentrations increased significantly between rest and the end of incremental exercise in the three groups ( $P < 0.05$ ) (Fig. 1). In the SCTAT and Cont groups, sVCAM-1 levels were not different from  $T_{\text{rest}}$  values during the recovery period. Conversely, in the SCT group, sVCAM-1 was still significantly higher at  $T_{1\text{h}}$  and  $T_{2\text{h}}$  than at rest ( $P < 0.05$ ). Compared with resting values, no significant differences were measured after incremental exercise in sICAM-1 and TNF- $\alpha$  plasma concentrations (Table 2).

No blood lactate intergroup difference was measured at rest (Fig. 2). Three minutes after incremental exercise, blood lactate significantly increased in the three groups, with SCT values being significantly higher than Cont values ( $P < 0.05$ ).

Table 1. Anthropometric characteristics

	Cont (n = 7)	SCT (n = 6)	SCTAT (n = 7)
Age, yr	29.5 $\pm$ 0.8	29.5 $\pm$ 2.7	29.4 $\pm$ 1.9
Weight, kg	74.1 $\pm$ 1.2	71.5 $\pm$ 2.5	72 $\pm$ 3.6
Height, cm	176.5 $\pm$ 1.9	176.5 $\pm$ 3.3	176.6 $\pm$ 2.1
$P_{\text{peak}}$ , W	240.4 $\pm$ 12.2	244.3 $\pm$ 8.9	261.5 $\pm$ 11.2
%HbA1	86.5 $\pm$ 0.5 $\dagger$	50.6 $\pm$ 0.4	54.6 $\pm$ 2.2
%HbA2	3.0 $\pm$ 0.2 $\dagger$	4.2 $\pm$ 0.4	4.2 $\pm$ 0.1
%HbF	1.2 $\pm$ 1.8	0.5 $\pm$ 0.1	0.6 $\pm$ 0.2
%HbS		38.3 $\pm$ 0.4	33.3 $\pm$ 0.4*

Values are means  $\pm$  SE.  $P_{\text{peak}}$ , maximal power. \*Significant difference between sickle cell trait (SCT) and SCT +  $\alpha$ -thalassemia (SCTAT) groups ( $P < 0.05$ );  $\dagger$  significant differences between control (Cont) group and the 2 other groups ( $P < 0.05$ ).

Table 2. Plasma concentration of sICAM-1 and TNF- $\alpha$  in Cont, SCT, and SCTAT groups at rest, at the end of incremental exercise, and 1, 2, and 24h after exercise

	Cont	SCT	SCTAT
sICAM-1, ng/ml			
T <sub>rest</sub>	489.1 $\pm$ 63.1	431.3 $\pm$ 97.4	291.3 $\pm$ 26.0
T <sub>ex</sub>	451.5 $\pm$ 50.9	418.6 $\pm$ 91.6	346.4 $\pm$ 34.8
T <sub>1h</sub>	408.6 $\pm$ 32.1	425.6 $\pm$ 91.5	329.3 $\pm$ 38.1
T <sub>2h</sub>	391.0 $\pm$ 44.6	386.8 $\pm$ 77.7	304.9 $\pm$ 30.5
T <sub>24h</sub>	398.6 $\pm$ 47.8	368.0 $\pm$ 70.2	280.4 $\pm$ 30.4
TNF- $\alpha$ , pg/ml			
T <sub>rest</sub>	3.2 $\pm$ 0.3	2.8 $\pm$ 0.2	4.6 $\pm$ 1.4
T <sub>ex</sub>	3.0 $\pm$ 0.3	2.5 $\pm$ 0.1	4.1 $\pm$ 1.4
T <sub>1h</sub>	2.7 $\pm$ 0.2	2.4 $\pm$ 0.2	4.5 $\pm$ 1.5
T <sub>2h</sub>	2.8 $\pm$ 0.1	3.0 $\pm$ 0.4	4.2 $\pm$ 0.9
T <sub>24h</sub>	2.7 $\pm$ 0.2	3.0 $\pm$ 0.3	3.2 $\pm$ 0.4

Values are means  $\pm$  SE. T<sub>rest</sub>, at rest; T<sub>ex</sub>, at end of incremental exercise; T<sub>1h</sub>, T<sub>2h</sub>, T<sub>24h</sub>, 1, 2, and 24 h after exercise; sICAM-1, soluble ICAM-1.

## DISCUSSION

The major aim of the present study was to assess whether the plasma concentrations of TNF- $\alpha$  and intercellular and vascular cell adhesion molecules differed between SCT athletes with or without coexisting  $\alpha$ -thalassemia trait and healthy athletes, both at rest and after a progressive and maximal exercise bout. The main findings of our study were that 1) before the exercise, resting sVCAM-1 was significantly higher in the SCT group compared with the other groups, but no differences occurred between the three groups in basal levels of sICAM-1 and TNF- $\alpha$ ; and 2) incremental exercise was associated with a significant rise in sVCAM-1 for all groups, which remained elevated up to 2 h after exercise in the SCT group only.

**Limitations.** As hematological variables are usually normal in SCT and SCTAT populations [RBC count, Hb levels (10, 27)], it has not been measured in the present study. However, the possibility of a weak anemia in some subjects might not be excluded and may explain the heterogeneity of some results.

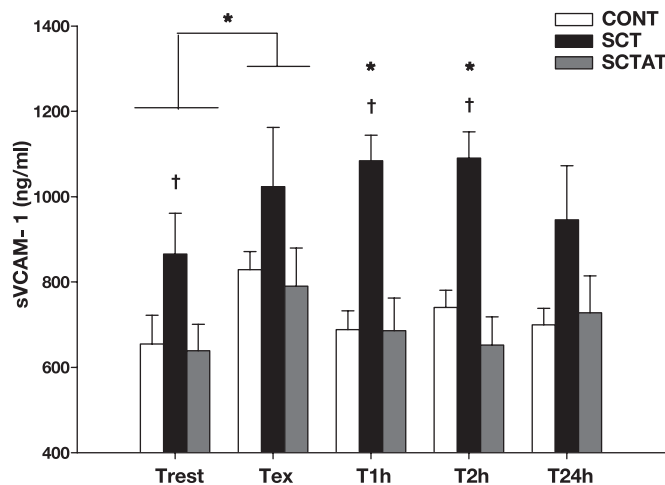


Fig. 1. Effect of incremental exercise on plasma levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) in the sickle cell trait (SCT), SCT and  $\alpha$ -thalassemia (SCTAT), and control (Cont) groups. T<sub>ex</sub>, immediately after completion of exercise protocol (T<sub>ex</sub>); T<sub>1h</sub>, T<sub>2h</sub>, and T<sub>24h</sub>, 1, 2, and 24 h after the end of exercise. \*Significant differences from at rest (T<sub>rest</sub>) ( $P < 0.05$ ); †significant differences between SCT and the 2 other groups ( $P < 0.05$ ). Values are shown as means  $\pm$  SE.

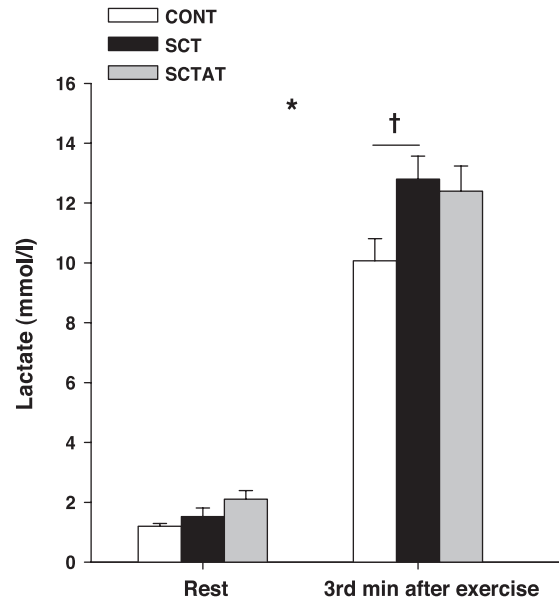


Fig. 2. Effect of incremental exercise on blood lactate in SCT, SCTAT, and control groups. \*Significant differences from T<sub>rest</sub> ( $P < 0.05$ ); †significant differences between SCT and control groups ( $P < 0.05$ ). Values are shown as means  $\pm$  SE.

**Basal inflammatory and adhesion molecules.** Athletes with SCT had higher sVCAM-1 in resting conditions compared with the other groups, although TNF- $\alpha$  concentrations were similar among the three groups. A variety of cells are able to effectively trap TNF- $\alpha$ , the result being that plasma TNF- $\alpha$  levels may not completely reflect synthesis by cells; this might explain the absence of plasma TNF- $\alpha$  increase in the present study (38). In addition, TNF- $\alpha$  is rapidly cleared from the circulation with generally peak values 8 h after strenuous exercise (31). The present findings partially support those of Duits and colleagues (13) who studied for the first time soluble adhesion molecule levels in subjects with SCT without any identifiable medical complications. They reported no alterations in sICAM-1 and cytokines in SCT compared with healthy controls subjects. However, these investigators also observed no changes in sVCAM-1, a finding that contrasts with our results. Further studies are needed to clarify the effects of SCT on this point in resting conditions.

The ligand for VCAM-1 that is expressed in stimulated endothelial cells is also the very late activation antigen 4 integrin (VLA-4) that is expressed on activated lymphocytes, monocytes, and reticulocytes (16). A greater adherence of sickle cells to endothelium regulated by VCAM-1 (3, 19) has been reported in microvascular studies particularly at low shear rate when flow characteristics of sickle erythrocytes under both oxygenated and deoxygenated conditions were altered (2, 19). Monchanin et al. (32) have recently demonstrated that SCT carriers are prone to increased blood viscosity and RBC rigidity and that such alteration may participate in the progressive decline in blood flow within microvascular beds and modifications in endothelial function (30, 40). Thus, although these hemorheological impairments do not seem to influence circulating cytokines as measured in the present study, increased blood viscosity could promote endothelial activation and adhesion of leukocytes and reticulocytes to VCAM-1 (41). This

vascular damage could in turn promote a slowing down of microvessel blood flow and even obstruct the vascular lumen (25). Nevertheless, adhesion phenomena result from complex interactions that may involve other plasma proteins such as thrombospondin and von Willebrand Factor. These proteins have been identified as bridging molecules mediating adhesion pathways between erythrocytes and endothelial cells (19). Thus we cannot exclude the possibility that hemorheological impairment identified in resting SCT individuals may affect pathways other than those mediated by the adhesion molecules studied herein.

**Effects of incremental exercise.** In the present study, maximal exercise performance was not affected by SCT and SCTAT, and  $P_{\text{peak}}$  values were in the range of  $P_{\text{peak}}$  found in the literature for these populations (10, 18). In all athletes, this exercise test did not affect the plasma concentrations of sICAM-1 and TNF- $\alpha$  as demonstrated by several previous studies using the same type of exercise (4, 31). However, plasma concentrations of sVCAM-1 were increased by 19.3%, 16.0%, and 19.2% for the Cont group, SCT group, and SCTAT group, respectively, immediately after incremental exercise ( $T_{\text{ex}}$ ) compared with resting values. This finding contrasts with Brevetti et al. (4) but concurs with the findings of Signorelli et al. (36), who reported an increase in sVCAM-1 in healthy subjects after incremental graded exercise. As previously shown, an increase in sVCAM-1 reflects an increase in endothelial VCAM-1 (35). Thus exercise performed until exhaustion appears to modify endothelial cell activation in all subjects, with a probable upregulation of endothelial VCAM-1. Despite a possible competition between soluble and endothelial VCAM-1 for blood cell receptors, one may hypothesize that VCAM-1 upregulation may promote the interaction between the endothelium and circulating blood cells (11, 36). Further study will have to verify the reality of endothelial VCAM-1 expression with exercise.

Of great interest were the differing changes in adhesion molecules that developed in SCT athletes with or without  $\alpha$ -thalassemia and Cont athletes during the recovery period after incremental exercise. Although sVCAM-1 returned to basal levels at  $T_{1h}$ ,  $T_{2h}$ , and  $T_{24h}$  for the Cont and SCTAT groups, sVCAM-1 remained significantly higher at  $T_{1h}$  and  $T_{2h}$  after the incremental exercise in the SCT group (Fig. 1). Interestingly, the high RBC rigidity recently reported in SCT carriers after incremental exercise (32) and after a short supra-maximal exercise (10) could impact on circulating adhesion molecules, particularly sVCAM-1. As suggested by works on ischemic vascular disease, modifications in endothelial function caused by physical stress may be associated with a worsening in hemorheological parameters particularly involving RBCs (15, 40). Several authors (3, 19) reported that endothelial activation, which is closely associated with adhesion of blood cells and subsequent vascular damage, was involved in vasoocclusive events in SCA patients. Indeed, the same phenomenon might be triggered under stressful physiological conditions in SCT athletes and might be a link with risk of sudden death reported several hours after exercise in these subjects (22). However, since TNF- $\alpha$  remained unchanged and constitutes the major promoter of VCAM-1 expression on endothelial cells (29), it is possible that alternative pathways leading to activation of adhesion molecules may occur in response to initiation of alternative, hitherto unidentified, path-

ways. Among the potential explanations for such observations, catecholamines (6, 33), other inflammatory molecules (5), and oxidative stress (12) could also underlie the excess induction of adhesion molecule concentrations as measured several hours after an acute maximal incremental exercise bout. Das et al. (12) suggested that exposure of SCT subjects to treadmill exercise resulted in an increase of RBC oxidation associated with relatively excessive production of reactive oxygen species. The latter likely plays a role in triggering dysfunctional endothelium, which inactivates nitric oxide production, a vasodilator, and the expression of adhesion molecules within the endothelium (28, 40). Further studies are required and should focus on the assessment of oxidative stress in SCT and SCTAT in response to different types of exercise.

Despite a recent report showing a similar exercise intolerance in SCT and SCTAT vs. control subjects (9), the absence of measurable differences in soluble adhesion molecules and TNF- $\alpha$  after exercise test between the Cont and SCTAT groups suggests that the presence of  $\alpha$ -thalassemia might be considered beneficial in SCT athletes, as initially proposed concerning endurance performance (26) and concerning blood viscosity (32). Further studies aiming to define the specific contribution of adhesion molecules in SCT carriers with and without  $\alpha$ -thalassemia are required to better understand vascular adhesion phenomena and could ultimately lead to novel approaches for prevention of microvascular end-organ injury in high-risk populations (1).

In conclusion, the present study demonstrated that, although Cont and SCTAT athletes exhibited similar basal plasma circulating levels of inflammatory and adhesion molecules, higher basal concentrations of sVCAM-1 were measured in SCT athletes. After an incremental exercise, sVCAM-1 was significantly increased in all subjects, and it remained elevated during 2 h only in the SCT group. Thus some risks for microcirculatory disturbances might not be excluded in some SCT athletes. Although this vascular dysfunction has never been demonstrated to induce vasoocclusive crisis in SCT subjects at rest and during exercise, this dysfunction could be amplified under stressful physiological conditions (exercise at altitude or with dehydration, for example). This phenomenon could raise the risk for vasoocclusion events several hours after exercise (3, 19, 21). Finally, and as previously suggested (32), the present findings underline the potential beneficial role of  $\alpha$ -thalassemia trait in the protection of SCT subjects from exercise-induced microcirculatory disturbances both at rest and after exercise.

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