Hemorheology, Sickle Cell Trait, and \( \alpha \)-Thalassemia in Athletes: Effects of Exercise

GERALDINE MONCHANIN\(^1\), PHILIPPE CONNES\(^1\), DIEU DONNE WOUASSI\(^2\), ALAIN FRANCINA\(^3\), BERNARD DJODA\(^2\), PIERRE EDMOND BANGA\(^2\), FRANÇOIS XAVIER OWONA\(^2\), PATRICE THIRIET\(^1\), RAPHAEL MASSARELLI\(^1\), AND CYRIL MARTIN\(^1\)

\(^1\)Center of Research and Innovation on Sports, Claude Bernard University of Lyon 1, Villeurbanne, FRANCE; \(^2\)National Institute of Youth and Sport, Yaounde, CAMEROON; and \(^3\)Molecular Pathology of Hemoglobin’s Unit, Edouard Herriot Hospital, Lyon, FRANCE

**ABSTRACT**


**Methods:** Six athletes with SCT (HbAS), 7 athletes with SCT and \( \alpha \)-thalassemia (HbASAT), and 10 control athletes (HbAA) performed a progressive and maximal exercise test on cycloergometer. Blood viscosity (\( \eta_b \)), plasma viscosity (\( \eta_p \)), \( \eta_b \) at corrected hematocrit (\( \eta_{b45} \)), hematocrit (Hct), and red blood cell (RBC) rigidity were assessed at rest, at maximal exercise and 24 h after exercise.

**Results:** \( \eta_b \) and \( \eta_p \) were not different between the three groups at any time. Exercise induced changes in \( \eta_b \) in HbAA and HbASAT groups but not in HbAS group. \( \eta_{b45} \) was higher in HbAS group compared with the other groups (\( P < 0.05 \)), at rest and 24 h after exercise and increased only in HbAA group in response to exercise. HbAS group had lower Hct than HbAA group at any time. Hct and \( \eta_p \) increased after exercise and declined under baseline values 24 h after exercise in all groups. RBC rigidity was higher in HbAS group compared with HbAA and HbASAT groups at any time, and was lower and higher at maximal exercise and 24 h after exercise, respectively, in all groups compared with resting values.

**Conclusions:** These results demonstrate that HbAS group is prone to higher RBC rigidity, which might lead to hemorheological alterations that are thought to participate to microcirculation disorders. However, these alterations are limited by the coexistence of \( \alpha \)-thalassemia. Moreover, hemorheological parameters were not further impaired in SCT athletes with or without \( \alpha \)-thalassemia in response to exercise. Training status might be protective from physiological stresses usually leading to sickling process in SCT carriers.

**Key Words:** HEMOGLOBINOPATHY, BLOOD VISCOSITY, RED BLOOD CELL DEFORMABILITY

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**Sickle cell disease (SCD)** is caused by the mutation of hemoglobin (Hb) A into HbS and mainly affects people of African descent. This mutation is caused by the substitution of a single amino acid, valine for glutamic acid. Sickle cell trait (SCT) carriers are characterized by the heterozygous presence of both HbA and HbS (AS genotype) in their red blood cells (RBC). The prevalence of SCD is around 20–40% in some areas of Black Africa and reaches 15% in European metropolises. In normal environmental conditions, RBC from subjects with SCD or SCT are less deformable than normal erythrocytes (AA genotype) (3). This hemorheological impairment is thought to participate to microcirculatory disorders reported in this population (21). If oxygen level is decreased, HbS polymerizes leading to a sickling process of erythrocytes that further alter their deformability (3,7). SCT has been associated with increased risk of sudden death during and several hours after intense exercise under normoxic and/or hypoxic conditions (20).

Exercise induces physiological changes (acidosis, dehydration, regional hypoxemia, or hyperthermia) that might promote sickling of HbS and blood rheological changes that might be responsible of vaso-occlusive events. However, blood rheology alterations may also occur in healthy athletes during and several hours after exercise (10,30), leading to a drastically increased blood viscosity (\( \eta_b \)) and decreased RBC deformability (5,30). Hemorheological parameters have never been investigated in SCT carriers in response to exercise, and it is still unknown whether changes in \( \eta_b \) and RBC deformability are more marked during and after exercise in SCT athletes than in healthy athletes.
Harkness (18) reported that the severity of SCT could be limited by the coexistence of SCT with α-thalassemia. The latter is caused by the deletion of a part of the gene encoding for the α-globin chain leading to a decreased production of the protein. The study of the coexistence of SCT and α-thalassemia is particularly interesting because of the high prevalence of both genetic defects in the black population (13). Le Gallais et al. (22) have hypothesized that coexistence of SCT with α-thalassemia could be protective in decreasing the severity of health complications caused by SCT alone because of blood rheology improvement in SCT carriers. It is now well admitted that there is a profound influence of the concentration of HbS on both the kinetics and the extent of HbS polymerization and thus on the clinical expression of SCD and SCT (19). Coexistence of α-thalassemia with SCT should be protective against the sickling process (18) because of decreased HbS intra-erythrocyte concentration. Indeed, hemorheological changes induced by acute exercise should be reduced in athletes with both SCT and α-thalassemia compared with athletes with SCT alone.

To test these hypotheses, we compared hemorheological parameters at rest, immediately at the end of a progressive and maximal exercise, and several hours after this exercise (24 h) between healthy trained subjects and well-trained athletes with SCT with or without α-thalassemia.

**METHODS**

**Subjects.** Six subjects with SCT (HbAS group), 7 subjects with both SCT and α-thalassemia (HbASAT group), and 10 controls (HbAA group) participated in the study after giving their informed consent. All subjects were competitive athletes and trained regularly for 10–15 yr in endurance sports (soccer and running). Athletes were recruited at the National Institute of Youth and Sport in Yaoundé (Cameroon) after a complete medical examination. Exclusion criteria included hypertension, stroke and malaria.

**Protocol.** The protocol was approved by the National Ethics Committee of Cameroon (registration number: 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 exercise on a cycloergometer until they reach maximal power (Ppeak). Venous blood samples were drawn from the antecubital vein of the nondominant arm at rest and after exercise to perform sickle cell trait and α-thalassemia detections, hemorheological measurements, and lactate concentration analysis. The laboratory was air-conditioned (room temperature 25–27°C, hygrometry 55%). No additional drink was allowed before and during exercise.

**Sickle cell trait and α-thalassemia detections.** Blood samples were collected at rest in EDTA tubes. The various Hb were isolated and quantified by an ion-exchange 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 with less than 50%. To study the presence of α-thalassemia, we used the technique from Chong et al. (8) with single-tube multiplex-PCR assay that is capable of detecting any combination of the six common single and double gene deletions in α-thalassemia. Mean values for Hbs were 38.3 ± 0.2% and 33.4 ± 0.1% in HbAS group and HbASAT group, respectively. The heterozygous form of α-thalassemia found in HbASAT group was marked by a deletion of 3.7 kb of DNA, containing one of the two linked α-globin genes (αα/α3.7).

**Exercise test.** Each subject performed a progressive and maximal exercise test on a mechanically braked ergometer (Ergomeca, France). The test began with a 5-min warm-up at 30 W. Explicit standardized instructions were given before each test. Pedaling speed remained constant (70 rpm) throughout the test, and a 30-W load was increased stepwise every minute until exhaustion (Ppeak).

**Blood and plasma analysis.** Blood for hemorheological measurements was sampled at rest; immediately at the end of exercise (Ppeak), that is, when subjects reached Ppeak and stopped exercise; and 24 h after the end of the test in EDTA tubes. To avoid RBC damage, hemorheological parameters were immediately measured after blood sampling. Measurements of ηb and ηp were performed with a cone plane viscometer (Brookfield DVII+, with CPE40 spindle), at high shear rate (375 s⁻¹). Under the present experimental conditions, the flow instability of the sample in the gap between the cone and the plate should not happen in this kind of viscometer (23). Hct was measured by a micro-method after blood microcentrifugation (Jouan Guetin-SA type 316).

Blood viscosity at corrected Hct (45%) (ηb₄₅) was calculated according to the equation of Quemada (26): 

\[
\eta_{b_{45}} = \eta_{r} \cdot \left(1 - \frac{1}{2} k \cdot Hct \right)^{-2}
\]

where k is a parameter that describes the rigidity of RBC at high shear rate that was calculated with the following equation:

\[
k = 2 \cdot \left(1 - \eta_{r}^{-0.5}\right) \cdot Hct^{-1}
\]

where \(\eta_{r}\) is relative blood viscosity (\(\eta_{b}/\eta_{p}\)).

Another index of RBC rigidity (Tk) was calculated at high shear rate (375 s⁻¹) according to the equation of Dintenfass (12):

\[
Tk = (\eta_{b_{10}} - 1)/(\eta_{b_{10}}^{0.4} \cdot Hct)
\]

Fingertip arterialized blood microsamples were taken for lactate analysis before beginning of pedaling and 3 min after the end of the test using a portable lactate analyzer (Lactate Pro LT-1710, Roche Bioelectronics, Switzerland, Basel).

**Statistical analysis.** Results are presented as mean ± SEM. Data related to subject characteristics and Ppeak were compared between the three groups using a one-way analysis of variance (ANOVA). Hemorheological parameters and blood lactate concentrations at rest, at the end of exercise, and 24 h after the end of the test were compared between all groups using a two-way ANOVA with repeated measurements on the intra factor. Pairwise contrasts were used when necessary to determine where significant differ-
Values are means ± SEM.

**RESULTS**

**Anthropometric characteristics and Ppeak.** As shown in Table 1, anthropometric data (age, weight, and height) and Ppeak achieved during the incremental exercise test were not different between the HbAA, HbAS, and in HbASAT groups.

**Blood and plasma analysis.** Hb was not statistically different among the three groups at any time (Table 2). Hb increased between rest and the end of exercise in the HbAA and HbASAT groups but did not change in the HbAS group. Hb was significantly decreased 24 h after exercise compared with resting values in all groups (P < 0.05)

ηp was not statistically different between the HbAA, HbAS, and HbASAT groups at any time (Table 2). Hct was lower in the HbAS group compared with the HbAA group at any time (P < 0.05; Table 2). Compared with resting values, Hct and ηp increased at the end of exercise (P < 0.05) and decreased 24 h after the exercise (P < 0.05) in the three groups.

When corrected to a 45% Hct, ηb (i.e., ηb45) was found to be statistically higher in the HbAS group than in the HbAA and HbASAT groups at rest and 24 h after exercise (P < 0.05; Fig. 1). No significant difference in ηb45 was found between the three groups at the end of exercise. Only the HbAA group exhibited a significant increase in ηb45 at the end of exercise (P < 0.05). No statistical difference was found between resting ηb45 and 24 h after exercise ηb45 in all groups.

Tk and k were higher in the HbAS group compared with the HbAA and HbASAT groups at rest, at the end of exercise, and 24 h after exercise (P < 0.05; Figs. 2 and 3). Changes in Tk and k in response to exercise, that is, immediately at the end of exercise and 24 h after the test, did not differ among the three groups. Tk and k were lower and higher at the end of exercise and 24 h after exercise compared with resting values, respectively (P < 0.05).

No difference in blood lactate concentration was found at rest (Fig. 4), but it increased significantly during exercise in all groups (P < 0.05). Three minutes after the end of exercise, the HbAS group had a higher value than the HbAA group.

A positive correlation between ηb45 and the percentage of HbS was found in the HbAS and HbASAT groups (r = 0.61; P < 0.05; Fig. 5a) while the percentage of HbS was negatively correlated to Hct (r = −0.636; P < 0.05; Fig. 5b). No significant correlation was found between the percentage of HbS and either ηb, ηp, Tk, or k.

**DISCUSSION**

The aim of this study was to determine whether hemorheological parameters of HbAS-trained athletes were affected at rest, at the end of a progressive and maximal exercise bout, and 24 h after exercise compared with HbAA-trained athletes and whether hemorheological alterations were less marked in HbASAT-trained subjects compared with HbAS-trained subjects. The main results of this study showed that 1) ηb45 was higher in the HbAS group compared with the HbAA and HbASAT groups at rest and 24 h after exercise; 2) Hct was lower in the HbAS group compared with the HbAA group at any time; 3) RBC rigidity indexes were higher in the HbAS group compared with the HbASAT and HbAA groups at any time; and 4) changes in hemorheological parameters between rest, the end of maximal exercise, and 24 h postexercise were different between the HbAS and the other groups.

In the present study, maximal exercise performance (Ppeak) was not affected by the presence of the sickle cell trait (Table 1). Ppeak from the HbAS group was comprised in the range of Ppeak measured by Gozal et al. (15) and confirms the findings of previous studies that showed similar maximal exercise power, during cycling exercise test, between HbAS athletes and HbAA athletes (27,28). Our results also demonstrated that Ppeak from athletes with both SCT and α-thalassemia was not different from Ppeak of athletes with normal Hb and athletes with SCT. These results are in contrast with those from Le Gallais et al. (22), who have hypothesized that endurance performance is improved in subjects with both SCT and α-thalassemia when compared with subjects with SCT alone. However, the exercise protocol used in this study might have been too short to really assess endurance capacity of the subjects. Further studies are needed to analyze cardiorespiratory responses of these subjects during prolonged and intense exercise bouts.

Blood lactate concentration was higher 3 min after the end of the exercise in the HbAS group compared with the two other groups. This finding is in accordance with the results from Freund et al. (14) but contrasts with those from Bileé et al. (2), Gozal et al. (15), and Sara et al. (27), who observed lower lactate levels in SCT carriers compared with HbAA subjects for a given intensity during a progressive and maximal exercise. These discrepancies are not well understood, but it is often reported that when HbAS and HbAA subjects are correctly matched in term of both physical performance and physical activity—as done in the studies from Bileé et al. (2) and Sara et al. (27)—HbAS subjects exhibit lower lactate levels during exercise. The results reported in the present study did not confirm these conclusions. Freund et al. (14) reported that SCT carriers were likely to produce more lactate than control subjects reaching exhaustion and/or to have an impaired ability to clear cir-
culating lactate. Although, the HbASAT group tended to have higher blood lactate concentration than the HbAA group, there was no statistically significant difference between these groups that might indicate the efficacy of α-thalassemia in limiting the blood lactate increase during exercise in SCT subjects. Further studies are required to better understand lactate metabolism in SCT carriers exhibiting or not α-thalassemia.

The most interesting results found in this study concern the different hemorheological pictures between SCT carriers with and without α-thalassemia and athletes with normal Hb. Although ηb measured at high shear rate was not different among the three groups at any time, ηb45 was higher in the HbAS group compared with the other groups at rest and after 24 h of recovery. This finding can be compared with those of Chien et al. (7), who reported that, even under conditions of complete oxygenation, the viscosity of HbSS blood exceeds the viscosity of HbAA blood at a same Hct. The positive correlation found between the percentage of HbS and ηb45 suggests that the severity of hemorheological impairment could be linked to the percentage of HbS.

At high shear rate, ηb mainly depends on Hct, ηp, and RBC rigidity. ηp was not significantly different between the three groups at rest and in response to exercise. To determine ηp, a rotational viscometer was used. Even if falling ball viscometer or capillary viscometer would have been more accurate to measure ηp (4), it has been shown that ηp could be determined with a rotational viscometer above a shear rate of 250 s⁻¹ (16,23). Moreover, Haidekker et al. (16) recently demonstrated that ηp values obtained with a new accurate technique were close to values obtained with a mechanical or rotational viscometer, similarly to the present study. One may assume that in vitro measurement of ηp with a cone plane viscometer was sufficiently accurate to be interpreted. Because a comparable value of ηp was found in HbAA and HbAS groups, RBC rigidity might be a key factor to explain higher ηb45 in subjects with SCT. The use of Dintenfass and Quemada’s equations (12,26) confirmed our hypothesis because Tk and k were higher in HbAS and HbASAT groups compared with HbAA group at any time. It might be advanced that altered membranes of HbAS erythrocytes and elevated internal viscosity of HbS have played a role (7).

The lack of difference in Tk, k, and ηb45 between the HbAA and HbASAT groups suggests that the presence of α-thalassemia in SCT carriers might be considered as beneficial for blood rheology as initially proposed by Le Gallais et al. (22). This result might be explained by the reduction in intra-erythrocyte HbS concentration in subjects with the coexistence of SCT and α-thalassemia compared with SCT subjects, as proved by the difference in HbS percentage between these two groups.

### Table 2. Hemorheological parameters at rest, at the end of maximal exercise, and 24 h after exercise in HbAA, HbAS, and HbASAT groups.

<table>
<thead>
<tr>
<th></th>
<th>HbAA (N = 10)</th>
<th>HbAS (N = 6)</th>
<th>HbASAT (N = 7)</th>
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<tbody>
<tr>
<td></td>
<td>At the End of Maximal Exercise</td>
<td>24 h after Exercise</td>
<td>At the End of Maximal Exercise</td>
</tr>
<tr>
<td>ηb (mPas) at 375 s⁻¹</td>
<td>4.78 ± 0.14</td>
<td>3.58 ± 0.19</td>
<td>5.01 ± 02</td>
</tr>
<tr>
<td>ηb (mPas) at 1250 s⁻¹</td>
<td>1.56 ± 0.02</td>
<td>1.69 ± 0.08</td>
<td>1.59 ± 0.06</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>46.02 ± 1.26</td>
<td>47.16 ± 0.98</td>
<td>41.75 ± 0.61</td>
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</tbody>
</table>

Values are means ± SEM. ηb, blood viscosity; ηp, plasma viscosity; Hct, hematocrit; * significant difference between rest, end of maximal exercise, and 24 h after exercise (P < 0.05); † significant difference between HbAA and HbASAT groups (P < 0.05); § significant difference between HbAA and HbAS groups (P < 0.05).

[FIGURE 1—Blood viscosity at corrected Hct (45%) at rest, at the end of maximal exercise, and 24 h after exercise in HbAA, HbAS, and HbASAT groups. * Significant difference between rest, end of maximal exercise and 24 h after exercise (P < 0.05); † Significant difference between HbASAT and HbAS groups (P < 0.05); § Significant difference between HbAA and HbAS groups (P < 0.05). Values are mean ± SEM.]

[FIGURE 2—Red blood cell deformability (Tk index) at rest, at the end of maximal exercise, and 24 h after exercise in HbAA, HbAS, and HbASAT groups. * Significant difference between rest, end of maximal exercise, and 24 h after exercise (P < 0.05); † Significant difference between HbAA and the other groups (P < 0.05); values are mean ± SEM.]

EXERCISE HEMORHEOLOGY IN SICKLE CELL TRAIT

Medicine & Science in Sports & Exercise
RBC deformability impairment in the HbAS group could exert detrimental effects on blood flow structuring in microcirculation (1,11,24,25). In the narrowest capillaries, whose luminal diameter is less than the undeformed size of RBC, erythrocytes advance in a greatly deformed state and in a single file flow. A thin blood plasma layer is always preserved during flow between the outer membranes of RBC and the luminal membranes of endothelial cells (25) and plays a lubricating role in preserving the blood flow in the lumina of microvessels. However, if RBC are less deformable, the plasma layer between RBC and endothelial membranes is reduced, leading to increased flow resistance (25). Moreover, less deformable RBC cannot pass through the narrowest capillaries or plug capillaries that favor plasma skimming (plasma flow without RBC) and lead to tissues ischemia (24). Indeed, SCT carriers could be prone to microvascular disorders because of the presence in their blood of low deformable RBC. However, the lower Hct observed in the HbAS group has partly compensated for the higher RBC rigidity found in this group leading to closed Hct in the three groups. Hct was negatively correlated with the percentage of HbS in the HbAS and HbASAT groups. It is well known that the risk for hemolysis is increased in subjects with poor deformable RBC, as it was the case in the HbAS group. Indeed, the lower Hct found in HbAS athletes could be due to the lack of RBC deformability, which might be partly caused by the presence of high percentage of HbS. If Hct from the HbAS group had been around values found in the HbAA and HbASAT groups, Hct increase would have been dramatic because hyperviscosity syndrome has been found to strongly participate to cardiovascular complications in other diseases (1,11). The possibility of one hemorheological parameter compensating for the abnormality of another is frequently reported in the literature and is considered as beneficial for cardiovascular health and for blood flow structuring in macrocirculation (6).

Changes in Hct and Hct between rest and the end of maximal exercise were similar in the three groups of athletes. Change in Hct during exercise has already been described in healthy athletes (5,9) and can be explained by
The decrease in $\eta_{45}$ in the HbAA and HbASAT groups may be explained by the decrease of $\eta_p$, which has partly compensated the increase in RBC rigidity. These findings are in accordance with previous studies that reported auto-hemodilution phenomenon and plasma volume expansion some hours after acute exercise (5) and alteration in RBC deformability 12 h after one exercise bout (30). The increase in RBC rigidity between rest and 24 h after exercise was not different between the HbAA and HbAS groups (8.9% and 6.1%, respectively). However, the decrease in $\eta_p$ between rest and 24 h after exercise was lower in the HbAS group than in the HbAA group (5.6% and 7.0%, respectively). Indeed, the drastically increase in RBC rigidity in the HbAS group was not entirely compensated by the small decrease in $\eta_p$. It seems that during recovery, SCT carriers are prone to higher hemorheological alterations than other groups. The reappearance and magnification of hemorheological alterations in SCT carriers some hours after one exercise bout might be implied in injuries and incidents frequently observed in this population (20,21).

In conclusion, blood rheological impairment was found at rest and 24 h after exercise in athletes with SCT alone. Thus, $\alpha$-thalassemia seems to protect SCT carriers against hemorheological impairment caused by the presence of SCT as previously suggested by Le Gallais et al. (22).

As already demonstrated by Brandao et al. (3), SCT carriers are prone to a reduced RBC deformability. These hemorheological alterations are thought to increase blood viscosity and participate to microcirculation disorders raising the risk for vaso-occlusion events. Surprisingly, in SCT carriers with or without $\alpha$-thalassemia, hemorheological parameters in response to exercise were not further altered compared with resting values. All subjects were well trained and it is well known that training modifies and improves hemorheological behavior at rest and in response to exercise (9,10). Indeed, the surprising lack of hemorheological impairment amplification in HbAS and HbASAT groups in response to exercise may simply be related to their training status.

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