Remodeling of skeletal muscle microvasculature in sickle cell trait and α-thalassemia

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Vincent L, Féasson L, Oyono-Enguéllé S, Banimbek V, Denis C, Guarneri C, Aufradet E, Monchanin G, Martin C, Gozal D, Dohbobga M, Wouassi D, Garet M, Thiriet P, Messonnier L.Remodeling of skeletal muscle microvasculature in sickle cell trait and α-thalassemia. Am J Physiol Heart Circ Physiol 298: H375–H384, 2010. First published November 13, 2009; doi:10.1152/ajpheart.00812.2009.—The influence of sickle cell trait and/or α-thalassemia on skeletal muscle microvascular network characteristics was assessed and compared with control subjects [hemoglobin (Hb) AA] in 30 Cameroonian residents [10 HbAA, 5 HbAA-αthalassemia (α-t), 6 HbAS, and 9 HbAS-αt] matched for maximal work capacity and daily energy expenditure. Subjects performed an incremental exercise to exhaustion and underwent a muscle biopsy. Muscle fiber type and surface area were not different among groups. However, sickle cell trait (SCT) was associated with lower capillary density (P < 0.05), lower capillary tortuosity (P < 0.001), and enlarged microvessels (P < 0.01). SCT carriers had reduced counts of microvessels <5-μm diameter, but a higher percentage of broader microvessels, i.e., diameter >10 μm (P < 0.05). α-Thalassemia seemed to be characterized by a higher capillary tortuosity and unchanged capillary density and diameter. Thus, while SCT is a priori clinically benign, we demonstrate for the first time that significant remodeling of the microvasculature occurs in SCT carriers. These modifications may possibly reflect protective adaptations against hemorheological and microcirculatory dysfunction induced by the presence of HbS. The remodeling of the microvascular network occurs to a lesser extent in α-thalassemia. In α-thalassemic subjects, increased capillary tortuosity would promote oxygen supply to muscle tissues and might compensate for the lower Hb content often reported in those subjects.

Because the larger RBCs, i.e., ~8–9 μm, have to pass through narrower capillaries (diameter ~4–5 μm) (49), the deformability of erythrocytes is a critical determinant of optimal blood flow in the microvasculature. Blood flow is also deeply affected by blood apparent viscosity (Poiseuille’s law). Several studies have thus far shown the presence of lower RBC deformability and higher blood apparent viscosity among SCT carriers compared with HbAA control (C) subjects (9, 18, 19, 47). Furthermore, the lower RBC deformability of SCT carriers could lead to endothelial damage, activation of endothelial cells, and thus promote endothelial cell-blood cell interactions, leading to inflammation. This proinflammatory state is mediated by an increased production of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (14) that results in an abnormal adhesion of the HbS-containing RBCs to the endothelium (7, 32). In accordance with this conceptual framework, Monchanin et al. (48) observed higher basal soluble VCAM-1 (sVCAM-1) concentrations in SCT carriers than in C subjects (9, 18, 19, 47). Furthermore, the lower RBC deformability of SCT carriers could lead to endothelial damage, activation of endothelial cells, and thus promote endothelial cell-blood cell interactions, leading to inflammation. This proinflammatory state is mediated by an increased production of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (14) that results in an abnormal adhesion of the HbS-containing RBCs to the endothelium (7, 32). In accordance with this conceptual framework, Monchanin et al. (48) observed higher basal soluble VCAM-1 (sVCAM-1) concentrations in SCT carriers than in C subjects (9, 18, 19, 47). Furthermore, the lower RBC deformability, higher blood apparent viscosity, and higher endothelial adhesion may 1) increase flow resistance in the microcirculation, 2) decrease blood flow, 3) raise the RBC transit time in the capillaries, 4) affect capillary recruitment and perfusion, and 5) favor plasma skimming in the capillaries, microcirculatory blood flow disorders are more likely to occur in SCT carriers (6, 13, 21, 31, 46). An immediate consequence of these microvascular blood flow alterations in SCT would include disturbances in oxygen delivery to the tissues (45, 51). In agreement with this hypothesis, Connes et al. (19) found a lower index of oxygen supply to peripheral tissues in SCT carriers. Conversely, the higher oxygen content in venous blood of SCT carriers during exercise (28) in the absence of SCD.

SICKLE CELL DISEASE IS A GENETIC abnormality of the red blood cell (RBC) due to a mutation of the gene that encodes for the synthesis of β-globin. The mutation consists in the substitution of the glutamic acid at the position β6 by one valin, leading to synthesis of abnormal hemoglobin (HbS). The properties of HbS differ drastically from those of HbA. The main difference lies in the fact that, in its deoxygenated form, HbS polymerizes and induces the sickling process of the RBCs (55). The sickle cell trait (SCT) is characterized by the heterozygous presence of both HbA and HbS (genotype AS). In SCT carriers, the proportion of HbS averages 34–38%, but may range between 20 and 45% of total Hb (<50%) (51, 56, 62). Although considered asymptomatic and benign, the SCT may be associated with morbidity and mortality (1, 17). In this context, fatal and nonfatal collapse after exercise, heat stroke and rhabdomyolysis have been repeatedly reported among SCT carriers (37, 38, 41, 52).

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any alterations in arterial blood oxygen tension could also be indicative of decreased oxygen supply to the tissues due to inadequate capillary blood flow.

Earlier studies on the microvascular network demonstrated that microvessels do not simply run straight along muscle fiber, but form sinuous pathways (4, 15). Because capillary tortuosity (CapTor) increases both flow resistance in the microcirculation and RBC transit time in the capillaries, and because HbS-containing RBCs are less deformable and adhere more to the endothelium, CapTor could worsen the risk for blood flow disorders and attendant deleterious consequences, i.e., inflammation and tissular hypoxia. Therefore, an adaptive mechanism that would aim at reducing the likelihood of microvascular dysfunction in SCT may consist in the presence of lower CapTor. Such adaptation would favor blood flow in the skeletal muscle microcirculation.

Tissue hypoxia and inflammation are postulated to serve as major stimuli of angiogenesis (14, 36, 40, 58). We, therefore, hypothesized that a more dense capillary network may be present in SCT carriers compared with C subjects. Such adaptation would 1) compensate to some extent for the putative lower CapTor, and 2) favor oxygen delivery to muscle fibers and promote metabolite exchange.

Thalassemias constitute another type of genetic mutation in Hb frequently observed in black populations. This abnormality results in an inadequate α- or β-globin chain-synthesis. Since HbS is a β-chain abnormality, SCT is regularly associated with α-thalassemia (SCTα-t). In several respects, α-thalassemia may afford a protective role in SCT carriers (20, 29). In those subjects with the dual hemoglobinopathy (SCTα-t), the percentage of HbS is generally <35% of total Hb, restricting the occurrence of HbS-related complications (20, 29, 59). In addition, α-thalassemia is also known to cause microcytosis (59), favoring the flow of RBCs through the capillary network. Thus α-thalassemia would be expected to dampen the hemorheological and microcirculatory alterations that were postulated above in reference to SCT carriers (20, 55, 60). In accordance with such hypothesis, RBC deformability and blood apparent viscosity were reported similar in SCTα-t and C subjects (47). Similarly, Monchanin et al. (48) observed that SCTα-t subjects exhibited analogous basal plasma levels of inflammatory (TNF-α) and adhesion molecules (ICAM-1 and VCAM-1) compared with C subjects.

The aim of the present study was to test the hypothesis that the presence of SCT, although asymptomatic, induces specific remodeling of skeletal muscle microvasculature, manifesting as lower CapTor and higher capillary density (CD), and that such changes would be absent in α-thalassemic subjects or attenuated in those who also harbor the SCT.

MATERIALS AND METHODS

Subjects

Thirty male SCT carriers and healthy (C) sedentary Cameroonian volunteers, with or without α-thalassemia, participated in the study. Subjects were then assigned into one of four distinct groups, i.e., C (n = 10), HbAA associated with α-thalassemia (AAα-t; n = 5), SCT (n = 6), and SCTα-t (n = 9). Age, height, and weight were 24 ± 1 yr, 173 ± 1 cm, and 67 ± 1 kg (mean ± SE), respectively. The study was conducted at the General Hospital of Yaoundé in Cameroon. The experimental protocol was approved by the local ethics committee (no. 10-12-2005) and was in accordance with the guidelines set by the Declaration of Helsinki for human studies. Before giving their written consent, all subjects were fully informed of the objectives and possible risks and/or discomforts of the experiments.

Recruitment was conducted by posted notices and word of mouth. Volunteers who 1) presented a hemoglobinopathy other than SCT and α-thalassemia, 2) suffered from a malaria bout within the past 2 mo, 3) took any medications, 4) were known human immunodeficiency virus carriers, and/or 5) took part in another research program, were excluded from the study. Smokers and regular alcohol drinkers were also excluded.

Experimental Design

The protocol included three visits consisting of 1) an inclusion protocol (visit 1); 2) an incremental exercise up to exhaustion (visit 2); and 3) a blood sampling, a muscle biopsy, and a physical activity questionnaire (visit 3).

Inclusion protocol. All prospective subjects underwent thorough physical examination, anthropometric measurements, blood sampling, and an incremental exercise to exhaustion. Blood samples were then assayed for HbA1, HbA2, HbS, and α-thalassemia genotype screening, as well as for determination of hematocrit, mean corpuscular volume (MCV), mean corpuscular Hb, mean corpuscular Hb concentration, RBC, white blood cell, and lymphocytes. Positive test results for SCT were determined by the presence of HbS at a level lower than 50% of total Hb using HPLC. The presence of α-thalassemia was detected with a single-tube, multiplex-PCR assay, capable of detecting any combination of the six common single and double gene deletions in α-thalassemia. Only one form of α-thalassemia was found in the present study, the heterozygous form marked by the deletion of 3.7 kb of DNA containing one of the two linked α-globin genes (αα/α3.7). The incremental exercise to exhaustion allowed the subjects to become familiar with the staff, experimental equipment, and testing procedures.

Incremental exercise to exhaustion. The subjects arrived at the testing site either at 8:00 AM or 12:00 PM. After a standardized breakfast or lunch, followed at least by 90 or 150 min of rest (respectively), subjects performed a graded exercise up to volitional exhaustion using a leg-cycle ergometer (Ketler, Ense-Parsit, Germany). The instantaneous power output and the pedaling frequency were delivered online by the computer device of the ergometer. The exercise started at 70 W. After 3 min of exercise at this load, the work rate increased by 35 W every 3 min thereafter. The exercise stopped when the subjects were no longer able to sustain the work rate and the required pedaling frequency set at 70 rpm. Heart rate (HR; beats/min) was measured continuously using a chest belt (Polar Electro, Kempele, Finland). Since a faulty oxygen sensor was used, it was not possible to measure whole body oxygen uptake, and, therefore, oxygen uptake data are not presented. This exercise session was used for determination of maximal HR (HRmax; beats/min) and the work rate associated with HRmax [maximal power (Pmax); W and W/kg].

Blood sample, muscle biopsy, and physical activity questionnaire. Subjects arrived at the test site, at either 8:00 AM or 12:00 PM. A 5-ml blood sample was then drawn for assay of soluble cell adhesion molecules and cytokines. Subsequently, the subjects were requested to lie down on a bed in the dorsal decubitus position. Muscle samples (respectively, from the vastus lateralis muscle of the left leg (at a level corresponding to one-third of the distance from the upper margin of the patella to the anterior superior iliac spin) using a Weil-Blakesley conchotome, according to the percutaneous technique (34). After shaving, asepsis was obtained using alcohol and iodized derivatives, and an incremental exercise to exhaustion using a leg-cycle ergometer (Ketler, Ense-Parsit, Germany). The instantaneous power output and the pedaling frequency were delivered online by the computer device of the ergometer. The exercise started at 70 W. After 3 min of exercise at this load, the work rate increased by 35 W every 3 min thereafter. The exercise stopped when the subjects were no longer able to sustain the work rate and the required pedaling frequency set at 70 rpm. Heart rate (HR; beats/min) was measured continuously using a chest belt (Polar Electro, Kempele, Finland). Since a faulty oxygen sensor was used, it was not possible to measure whole body oxygen uptake, and, therefore, oxygen uptake data are not presented. This exercise session was used for determination of maximal HR (HRmax; beats/min) and the work rate associated with HRmax [maximal power (Pmax); W and W/kg].

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mostasis was then ensured by a 5-min compression, and the access closed by sterile strips. Part of the biopsy sample containing well-identified fascicles was oriented under a stereo microscope and mounted in Cryomount (Histolab, Göteborg, Sweden), then frozen in isopentan, and stored in liquid nitrogen until histochemical and immunohistochemical analyses. This component of the biopsy sample was used for determination of different muscle fiber-type and microvascular network analysis. A physical activity questionnaire estimating the daily energy expenditure (DEE) was administered to the subjects (24).

**Soluble Cell Adhesion Molecules and Cytokines Measurements**

For adhesion molecules, venous blood was collected in EDTA tubes. Plasma was separated by centrifugation (10 min at 3,000 g) and stored at −80°C until analysis. The measurement of sVCAM-1, soluble (s) ICAM-1, sE-selectin, and sP-selectin was performed using commercially available ELISA kits (Diaclone Systems, Besançon, France), according to the manufacturer’s instructions. All samples were determined in duplicate, and the sensitivity limits were as follows: 0.6 ng/ml (sVCAM-1), 0.1 mg/ml (sICAM-1), 0.5 ng/ml (sE-selectin), and 1.06 ng/ml (sP-selectin). 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 (57).

Cytokines were quantified at rest using a Luminex fluorescent bead-based immunoassay kit (Luminex, Austin, TX) to simultaneously measure interleukin (IL)-1β, IL-4, IL-8, and IL-10. The R&D Fluorokine MAP Human Cytokine Multiplex Panel was used for this purpose (R&D Systems). The detection limits were, respectively, 64, 1, 16, and 16 pg/ml.

**Muscle Fiber, Cytochrome-C Oxidase Activity, and Microvascular Network Analyses**

**Immunocytochemical and histochemical assays.** Cryostat serial transverse sections of 10 μm thick were cut using a microtome at −20°C (HM 560, Microm, Walldorf, Germany). Fiber-type distribution was studied on serial sections stained for myofibrillar ATPase after preincubation at different pH (pH 4.35 and 4.55), according to the methodology of Brooke and Kaiser (11). Fiber-type distribution was also studied on immunohistochemical serial preparations using anti-fast Ia myosin heavy chain N2.261 (Alexis Biochemicals) and anti-slow myosin heavy chain A4.951 (Alexis Biochemicals) monoclonal antibodies. The fibers were designated as I, Ia, and IIx (previously referred to as Iib by Brooke and Kaiser). For cytochrome-c oxidase (COX) activity, measurements were performed by converting the image to gray scale to determine optical density using SigmaScan Pro software (SPSS Science, Chicago, IL). The mean relative optical density per pixel was determined by subtracting the optical density of the background. For each subject, a single value per structure was obtained by averaging measurements from a mean of 150 well-identified muscular fibers.

**CD.** In a given area, the number of capillaries was counted, and the CD was expressed as the number of capillaries per square millimeter (cap/mm²).

**Microscopy and analyses.** Muscle sections were viewed under a light microscope (Eclipse E400, Nikon, Badhoevedorp, the Netherlands) connected to a digital camera (Coolpix 990, Nikon). Photographs were taken at ×40 to ×400 magnification for 1) muscle fiber-type determination, fiber surface area and perimeter (PF), 2) COX activity, and 3) CD. For these analyses, an average of eight fields were examined in each section, allowing for inspection of ~70 fibers per individual sample (see Ref. 15). Morphological and morphometric analysis of microvessels, i.e., capillary outer diameter (COD), capillary perimeter (CP), capillary surface area (CSA), and length of contact between a capillary and a muscle fiber (LC), were performed at high magnification (a ×1,000 optical magnification coupled with a ×2.6 digital magnification) to zoom in on one microvessel. In this case of microvessel morphometric assessments, an average of 14 fields per section were examined, corresponding to the analysis of ~150 microvessels per slice (12). All photographs were analyzed using SigmaScan Pro 5.0 software (SPSS Science, Chicago, IL), which permits accurate delineation of the muscle membrane and the microvessel wall with a line tracking tool and, therefore, enables collection of 1) muscle PF and surface area and 2) CP, CSA, and CD measurements simultaneously. The LC were obtained using the distance item of the line-tracking tool. Measurement accuracy was ensured for each photograph by calibrating the distance value with a surveyor’s rod.

**COX activity.** Measurements of COX intensity were performed by converting the image to gray scale to determine optical density using SigmaScan Pro software (SPSS Science, Chicago, IL). The mean relative optical density per pixel was determined by subtracting the optical density of the background. For each subject, a single value per structure was obtained by averaging measurements from a mean of 150 well-identified muscular fibers.

Morphological and morphometric analysis of the microvascular network. COD was classified as being <5 μm, between 5 and 10 μm, and >10 μm. In the present study, most of the microvessels counted in the endomysium presented a diameter <10 μm. However, even within this diameter range, some microvessels might represent precapillary arterioles (27). Thus the criterion of 10-μm diameter proposed by Charifi et al. (15) is not sufficient for distinction between a capillary and a precapillary arteriole. For this reason, it seemed appropriate to partition these vessels into two categories, namely, <5 and >5 μm. CP and CSA were also measured. The immunostaining of microvessel walls allowed visualization of all portions of transversally or longitudinally running microvessels and measurement of the LC between the microvessels and the fibers.

**Microvessel/fiber interface.** As proposed by Sullivan and Pittman (61) and later by Charifi et al. (15), the ratio between LC and the PF was calculated (LC/PF). This ratio expresses the percentage of the circumference of a muscle fiber in contact with capillaries and gives information on the diffusion capacity between the capillary network and the muscle fibers.

**CapTor.** In a previous study, it was suggested that the LC/PF is an adequate index of the CapTor (15). This is correct, if there are no differences in CD, fiber and/or CSA, and capillary diameters among the compared groups. However, in the present study, due to the marked differences among our groups in the capillary dimensions, LC/PF would not be appropriate as an index of tortuosity. Thus, microvascular network anisotropy was assessed using the following equation:

\[
\text{CapTor} = \frac{\text{CSA}}{\pi (\text{COD}/2)^2}
\]

(1)

As presented in Fig. 1, the more the ratio is elevated (i.e., >1), the more the capillary is tortuous. A ratio of 1 describes a microvessel that is perfectly perpendicular to the muscle section.
Other functional indexes related to the microvascular network. Our analysis of the microvascular network further relied on other different functional indexes that were derived from the measurements using the classical morphometric assumption that, in a cross-sectional histological section, length is related to area and area to volume. These additional functional indexes were calculated as follows: 1) the product of CD and CP (CD \times CP) denotes the capillary surface (per mm²) available for gaseous and metabolite exchanges with interstitial fluids (modified from Ref. 36), and 2) the product of CD and CSA (modified from Ref. 36) estimates the instantaneous volume of blood that crosses the 10-μm-thick section (CD \times CSA).

Statistical Analysis

Descriptive statistics are expressed as means ± SE. A two-way ANOVA was used to determine the effects of HbS, α-thalassemia, and of the interaction of these two factors on the different measured parameters, unless indicated otherwise. Differences between values were considered to be significant for P ≤ 0.05 and to represent a tendency for 0.05 < P ≤ 0.10.

RESULTS

Physiological Characteristics and Physical Activity

Because physical fitness can affect the capillary network, we ascertained that C subjects and sickle trait patients, with or without α-thalassemia, were matched for DEE (assessed by our questionnaire) and exercise capacity (assessed by the P_max obtained during an incremental exercise up to exhaustion). Table 1 shows that there were no differences among the different groups for either DEE or P_max. None of the subjects experienced adverse events during or after incremental exercise. At exhaustion, HR_max reached 181 ± 3 beats/min.

Hematological Data

The percentages of HbA1 and HbA2 were, respectively, higher and lower in the C group compared with the two SCT groups (Table 1). The percentage of HbS was significantly higher in the SCT group compared with the SCTα-t group (P < 0.05). Lower MCV, mean corpuscular Hb, and mean corpuscular Hb concentration values were measured in α-thalassemia, confirming the microcytosis classically reported for this population. RBCs were higher in α-thalassemia, MCV was slightly lower in SCT. White blood cell and lymphocyte counts were not statistically different among the four groups.

Muscle Characteristics

The distribution of muscle fiber types was 33.6 ± 2.4, 52.0 ± 2.5, and 14.4 ± 2.1% for type I, Ila, and IIX, respectively.

Table 1. Some anthropometric and physiological characteristics and hematological data of the subjects

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Values are means ± SE; n, no. of subjects; C, control [hemoglobin (Hb) AA]; AAα-t, hemoglobin (Hb) AA associated with α-thalassemia; SCT, sickle cell trait (HbAS); SCTα-t, SCT associated with α-thalassemia; SBP and DBP, systolic and diastolic blood pressure, respectively; HR_max, maximal heart rate; P_max, maximal power; DEE, daily energy expenditure; Hct, hematocrit; MCV, mean cell volume; MCH, mean cell Hb; MCHC, mean cell Hb concentration; RBC and WBC, red and white blood cell, respectively; N/A, none applicant; NS, nonsignificant.
tively. No fiber-type distribution differences were detected among the four groups (Table 2); of note, IIa fibers account for more than one-half of the fiber population. Muscle fiber surface area was also similar among the groups (Table 2). COX activity (Table 2) was significantly lower in type IIa fibers (the most important population of muscle fibers) and tended strongly to be lower in type I (P = 0.0505) and IIx fibers (P = 0.0637).

Microvascular Network: Quantitative Analysis

Figure 2 shows photographs of a typical microvascular network in HbAA (C), AAτ-t, HbAS (SCT), and SCTα-t subjects. Two-way ANOVA showed that the presence of SCT was associated with a lower CD (Fig. 3A).

Microvessels: Morphological and Morphometrical Analysis

The COD was significantly higher in subjects with HbS-containing RBCs (Fig. 3B). However, COD was not altered by α-thalassemia (nonsignificant). SCT was also associated with reduced percentage and number of the narrowest capillaries (<5 μm) and with higher percentage and number of wider microvessels (>10 μm) (Fig. 4). The presence of α-thalassemia was not associated with any of such changes. However,
microvessel perimeter (Table 2) and surface area (Fig. 5A), while SCT did not. Furthermore, CapTor was lower in SCT (Fig. 3C). CapTor tended also to be higher in α-thalassemic subjects (P < 0.1). Note that the Aα-t group showed a significantly higher CapTor than the three other groups (t-test: P < 0.05, P < 0.001, and P < 0.001 for C, SCT, and SCTα-t, respectively; Fig. 3C).

Microvessels/Muscle Fibers Interface

Although the mean values of LC and LC/PF were higher in α-thalassemic subjects, this difference did not achieve statistical significance (Table 2). LC and LC/PF were not altered in the presence of SCT (Table 2).

Other Functional Indexes

The estimated surface area available for gas and metabolite exchange, which is represented by the product of CD × CP, was not different between the four groups (Table 2). In addition, an estimate of blood volume (CD × CSA) in the 10-μm-thick cut tended to be higher in subjects with α-thalassemia (Fig. 5B).

Adhesives Molecules and Cytokines Plasma Concentrations

sICAM-1, sVCAM-1, sE-selectin, sP-selectin, IL-8, and IL-10 concentrations were similar among the four groups. However, plasma concentrations of IL-4 tended to be lower in SCT carriers (Table 3).

DISCUSSION

The present study is the first to describe the remodeling of the skeletal muscle microvasculature associated with the presence of the SCT and α-thalassemia. The main findings were that SCT carriers, although asymptomatic, display lower CD and tortuosity in the presence of more frequent wider microvessels, while α-thalassemia does not alter CD and dimensions, but tends to increase microvessel tortuosity. Of particular note, these findings followed careful matching of the various subject groups for maximal exercise work capacity as well as for DEE. Therefore, the skeletal muscle microvascular adaptations in SCT and α-thalassemia cannot be explained on the basis of preexisting differences in physical fitness and/or by more or less active lifestyles, which are known to modify the microvascular network (2, 36, 53).

SCT

Contrary to our original hypothesis, SCT was associated with a lower CD (Fig. 3A) that was not explained by the presence of a larger muscle fiber surface area (Table 2). Thus this likely reflects a true reduction in the density of the capillary network in SCT carriers. Although the underlying
Taken together, these first results suggest higher capillary dimensions in SCT carriers. In accordance with such hypothesis, the mean capillary diameter (COD) was found enhanced in SCT subjects compared with HbAA counterparts (Fig. 3B). Furthermore, Fig. 4A shows clearly that the percentages of the narrowest (<5 μm) and widest (>10 μm) measured microvessels are, respectively, lower and higher in SCT carriers than in C subjects. Because SCT carriers display a lower CD, the higher percentage of the widest microvessels does not necessarily result in a higher number per square millimeter of microvessels larger than 10 μm in the SCT carriers. Nevertheless, our results show clearly that the number of wide microvessels per square millimeter is higher in SCT carriers than in C subjects (Fig. 4B). This particular adaptation of the SCT carrier microvasculature may be attributed to either 1) a regression of the narrowest microvessels, 2) an enlargement (of the lumen but also of the capillary wall) of the existing microvessels, 3) an arterIALIZation of some capillaries, 4) a neovascularization, 5) a bridging of the stenosis area with bypasses (enlarged collateral blood vessels), or 6) a combination of all aforementioned possibilities (36). Among the possible mechanisms, the particular hemorrheology and hemodynamics of the sickle cell carriers might stimulate matrix metalloproteinases, which play an important role in the vessel matrix digestion and internal elastic lamina reorganization, contributing to vessel distensibility and resulting directly in vessel diameter enlargements (42). Besides, the enlargement of the SCT carrier microvasculature diameter occurs in the presence of lower IL-4, one of the known inhibitors of angiogenesis (Table 3). We would again wish to reemphasize that the potential underlying mechanisms leading to the observed enlargement of the microvessels diameter remains speculative at best. Notwithstanding such considerations, the microvascular adaptations in SCT carriers should facilitate RBC passage through the capillary network and thus reduce the likelihood of local blood flow alterations, inflammation, and abnormal adhesion to the endothelium, and this assumption is in agreement with the normal sICAM-1, sVCAM-1, sE-selectin, sP-selectin, sIL-4, IL-8, and IL-10.

### Table 3. Plasma concentrations of sVCAM-1, sICAM-1, sE-selectin, sP-selectin, IL-4, IL-8, and IL-10

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>AAo-t</th>
<th>SCT</th>
<th>SCTo-t</th>
<th>HbS</th>
<th>α-Thalassemia</th>
<th>Crossed</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>5</td>
<td>6</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sVCAM-1, ng/ml</td>
<td>1.522±0.277</td>
<td>1.495±0.262</td>
<td>1.833±0.185</td>
<td>1.383±0.164</td>
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</tr>
<tr>
<td>sICAM-1, ng/ml</td>
<td>472.5±80.2</td>
<td>380.2±60.9</td>
<td>489.9±118.5</td>
<td>465.1±85.1</td>
<td></td>
<td></td>
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<tr>
<td>sE-selectin, ng/ml</td>
<td>61.5±6.6</td>
<td>70.7±20.3</td>
<td>53.6±11.9</td>
<td>58.8±7.4</td>
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<tr>
<td>sP-selectin, ng/ml</td>
<td>140.3±31.2</td>
<td>83.8±22.6</td>
<td>157.8±54.6</td>
<td>176.1±85.4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IL-4, pg/ml</td>
<td>374.0±114.4</td>
<td>198.7±45.6</td>
<td>162.8±67.5</td>
<td>0.0656</td>
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<tr>
<td>IL-8, pg/ml</td>
<td>89.9±43.5</td>
<td>N/M</td>
<td>69.9±22.4</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>IL-10, pg/ml</td>
<td>53.5±15.5</td>
<td>N/M</td>
<td>43.3±13.7</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. sVCAM-1, sICAM-1, sE-selectin, sP-selectin: soluble VCAM-1, ICAM-1, E-selectin, and P-selectin, respectively; IL, interleukin; N/M, not measured.
IL-8, and IL-10 concentrations in SCT carriers found in the present study (Table 3).

An alternative explanation could be that the enlargement of the microvessels, in fact, serves to compensate for the lower CD and thus allows for maintaining blood volume in the microvasculature. In accordance with this assumption, the product CD × CSA that denotes existing microvascular blood volume, albeit very imperfectly (35, 44, 63), was not different in SCT carriers compared with HbAA subjects (Fig. 5B).

The observed enlargement of the skeletal muscle microvasculature in SCT carriers is reminiscent of similar findings among patients with coronary heart disease. Indeed, blood is reoriented in these patients through enlarged collateral blood vessels that bypass the occlusion (14, 23, 33). Along the same lines, perfusion of animal muscle with human HbS-containing RBCs resulted in reduced microvascular flow, and the latter was compensated by vasodilatation with shunting of blood through vascular pathways running parallel to the capillaries occluded by RBCs (43).

CapTor can basically be considered as an enlargement of the capillary surface (3, 5). As expected, microvessel tortuosity was lower in SCT carriers (Fig. 3C). By reducing the transit time of the RBCs in the capillary, the lower CapTor of the SCT carriers may constitute a protective mechanism against blood flow alterations. Thus enlargement of the tortuous microvessels even in healthy subjects could prevent rheological problems that may develop in tortuous capillaries (4).

It is important to keep in mind that the dynamic changes in the microcirculation (i.e., vasoconstriction/vasodilatation of resistance vessels, and perhaps dynamic changes of the dimension of capillaries) that occur in vivo can have even greater impacts on tissue perfusion compared with static changes in the microvascular dimensions. Therefore, the inferences drawn from our observations, made ex vivo, to in vivo conditions should be viewed with caution. Nevertheless, taken together, the lower CD and tortuosity, along with enlargement of the microvessels in SCT subjects, supports the notion that, although subclinical, hemorheological disturbances do occur in SCT and are sufficient to promote structural vascular adaptations.

**α-Thalassemia**

The present study shows that α-thalassemia is associated with higher CP and area (i.e., CP and CSA, respectively) without alterations in capillary diameter (i.e., COD) (Table 2, Fig. 3B, and Fig. 5A). These findings suggest a higher microvasculature tortuosity in α-thalassemic subjects. This inference is also supported by the fact that the Aααt group showed a significantly higher CapTor than the other groups (Fig. 3C). The product CD × CSA, which provides indirect information on the instantaneous surface of blood per square millimeter, also trended toward higher values in subjects with α-thalassemia, seemingly indicating improved blood supply in the microcirculation (Fig. 5B). In healthy subjects, these adaptations would be expected to facilitate the supply of oxygen to the muscular tissue and the efflux of metabolites, such as lactate, from the muscle (4). However, inferences between such vascular changes and enhanced oxygen supply to the tissues are not so obvious (26), especially in α-thalassemic subjects who suffer often (although not the case in the present study) from lower total Hb, and, as such, higher blood supply could, at least partially, compensate for the lower Hb and allow maintenance of oxygen supply to the muscles in those latter subjects. Besides, the RBCs microcytosis observed in α-thalassemic subjects might also prevent hemorheological troubles that might occur in tortuous capillaries.

**Dissociated Mechanisms**

Several studies report that a dense capillary network is often associated with tortuous capillaries that also exhibit larger diameters. Capillaries in red muscles are wider and more tortuous than the capillaries in white muscles (4). Hassler and Stroinski-Kusinova (30) found a more dense vascular network with more tortuous and wider capillaries in hypertrophic rat muscles. Charifi et al. (15) found that endurance training in elderly improves capillary network and tortuosity. The lower CD and tortuosity but wider capillary diameters reported herein in SCT and the higher CapTor but unchanged CD and diameters in α-thalassemia would argue in favor of the hypothesis that the underlying mechanisms that regulate either CD, tortuosity, or diameter are not the same. In other words, the occurrence of divergent and flexible rather than only confluent adaptations of CD, tortuosity, and diameter is possible.

**Conclusion**

Although SCT is considered a clinically benign condition, we demonstrate for the first time evidence for significant remodeling of the microvasculature in SCT carriers. The adaptive remodeling is characterized by a lower CD, a lower CapTor, a wider mean capillary diameter, reduced numbers of narrow capillaries, and increased numbers of wide capillaries, most likely as a concerted effort to promote local blood flow in the microcirculation of the SCT subjects and compensate for blood apparent viscosity and reduced RBCs deformability. Furthermore, we also show evidence that α-thalassemia induces a higher CapTor that may serve to compensate for lower Hb content and thus help maintain oxygen supply to muscle tissues.

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**DISCLOSURES**

No conflicts of interest are declared by the author(s).

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