Effects of regular physical activity on skeletal muscle structural, energetic, and microvascular properties in carriers of sickle cell trait

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Vincent L, Oyono-Enguillé S, Féasson L, Banimbek V, Dohbobga M, Martin C, Thiriet P, Francina A, Dubouchaud H, Sanchez H, Chapot R, Denis C, Geyssant A, Messonnier L. Effects of regular physical activity on skeletal muscle structural, energetic and microvascular properties in carriers of sickle cell trait. J Appl Physiol 113: 549–556, 2012. First published June 14, 2012; doi:10.1152/japplphysiol.01573.2011.—To assess the effects of regular physical activity on muscle functional characteristics of carriers of sickle cell trait (SCT), 39 untrained (U) and trained (T) hemoglobin (Hb)AA (CON) and SCT subjects (U-CON, n = 12; U-SCT, n = 8; T-CON, n = 10; and T-SCT, n = 9) performed a graded exercise and a time to exhaustion (TeX) test, and were subjected to a muscle biopsy. Maximal power, total work performed during TeX, citrate synthase and cytochrome c oxidase (COX) activities, respiratory chain complexes I and IV content, and capillary density (CD), diameter (COD), and surface area (CSA) were upregulated by the same proportion in T-CON and T-SCT compared with their untrained counterparts. These proportionally similar differences imply that the observed discrepancies between U-SCT and U-CON remained in the trained state.

Controversies exist whether carriage of sickle cell trait is a risk factor during physical activity. On the one hand, some researchers (6, 26, 37, 45) argue that SCT can be considered asymptomatic and a benign condition during physical activity. Moreover, several studies (5, 30, 39) have shown that carriers of SCT may present predispositions for performance during very short supramaximal exercise. On the other hand, some researchers (11, 21, 33) suggest that carriers of SCT should be considered at risk during exercise. Indeed, an increasing number of studies (2, 9, 16, 17, 18, 19, 20, 22, 32, 33, 42, 47, 51, 54, 58) report nonfatal and fatal collapses, heat stroke, and rhabdomyolysis after exercise in subjects carrying SCT. Part of the severe exercise-related complications observed in carriers of SCT might be explained by hemorheological disorders, i.e., increased blood apparent viscosity and vascular adhesion, decreased RBC deformability, sickling, increased intravascular coagulation, and higher oxidative stress (4, 13, 14, 40, 43, 44, 48, 55).

Although similar in terms of maximal aerobic performance (12), muscle fiber type distribution, and key enzyme activities of glycolytic or tricarboxylic acid cycle pathways (56), we (50) recently reported that compared with HbAA counterparts, carriers of SCT displayed particular muscle functional characteristics including 1) a trend toward a higher type IIx fiber surface area, 2) a significantly lower cytochrome c oxidase (COX) activity in type IIa fibers while similar trends were observed in type I and IIx fibers, and 3) a trend toward a lower creatine kinase (CK) activity, an enzyme also known for playing a key role in oxidative energy metabolism. However, the main changes observed in carriers of SCT consisted in their profound skeletal muscle microvasculature remodeling characterized by lower capillary density (CD) and tortuosity and a general enlargement of microvessels (55). Taken together, these latter results seem to indicate that carriers of SCT might...
be prone to impaired oxygen supply to and utilization by tissues that corroborates the results of Connes et al. (13).

It is well established that many structural and biochemical components of the skeletal muscle are markedly upregulated by regular physical activity (24, 27, 31). For instance, endurance training increases CD in all fiber types (1, 7, 31, 41) and modifies capillary geometry (10, 46) that improves O2 delivery to muscular tissue. Likewise, mitochondrial content (28, 34) and oxidative enzyme activities, including COX (1), are both increased by endurance training. Unfortunately, the effects of regular physical activity on structural and energetic characteristics of carriers of SCT have never been investigated.

Therefore, the aim of the present study was to assess the effects of regular physical activity on functional and energetic muscle characteristics of carriers of SCT.

MATERIALS AND METHODS

Subjects

Thirty-nine male Cameroonians volunteered to participate in the study. Subjects were allocated into four groups according to their habitual physical activity and their hemoglobin status: untrained HbAA controls (U-CON; n = 12), untrained carriers of SCT (U-SCT; n = 8), trained HbAA controls (T-CON; n = 10), and trained carriers of SCT (T-SCT; n = 9). Untrained subjects were sedentary subjects who reported no regular physical activity for the 2 previous years while trained subjects were collegiate soccer players (~8 h/wk for several years). Age, height, and weight were 24 ± 1 y, 173 ± 1 cm, and 66 ± 1 kg (means ± SE). The study took place at the General Hospital of Yaounde (Cameroon). These experiments were approved by the Ethics Committee of the Faculty of Medicine of University of Yaounde 1 (no. 10–12-2005) and were 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, possible discomforts, and potential benefits of the experiments. Part of the results has been published elsewhere for other purpose (55, 56).

Experimental Design

The experimental protocol included four visits performed a week apart.

Visit 1: inclusion protocol. Subjects were submitted to a thorough physical examination, anthropometric measurements, and blood samplings. Blood samples were drawn from the antecubital vein of the nondominant arm at rest for HbA1, HbA2, HbS, and hemoglobin quantification twice by HPLC. Positive test results for SCT were checked a posteriori (see MATERIALS AND METHODS). Untrained and trained carriers of SCT (55, 56) were not considered a priori, and therefore, the exclusion criteria in the present study were: male sex; age older than 30 yr; 1 cm, and stored in liquid nitrogen until histochemical and immunohistochemical analyses. This part was used for determination of different muscle fiber type distribution. The remainder of the sample was rapidly frozen and stored in liquid nitrogen until analysis of enzymes activities and content of the different mitochondrial respiratory chain complexes.

Visit 2: incremental exercise to exhaustion. The exercise session was performed on a bicycle ergometer (Ketler, Ense-Parsit, Germany). 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). This exercise session was used for determination of maximal HR (HRmax; beats/min) and the work rate associated with HRmax (Pmax; W and W/kg), which was estimated by linear interpolation from the HR vs. work rate curve.

Visit 3: muscle biopsy. A week later, subjects arrived at the hospital either at 8:00 AM or 12:00 PM. Subjects were placed on a bed in the dorsal decubitus position. A small incision was made in the skin and fascia under local anesthesia. A biopsy of the vastus lateralis muscle was taken with a Wel Blakesley forceps at rest. Part of the biopsy sample containing well-identified fascicles was oriented under a stereo microscope and mounted in cryomount and then frozen and stored in liquid nitrogen until histochemical and immunohistochemical analyses. This part was used for determination of different muscle fiber type distribution. The remainder of the sample was rapidly frozen and stored in liquid nitrogen until analysis of enzymes activities and content of the different mitochondrial respiratory chain complexes.

Visit 4: time to exhaustion at 110% P0217. The third week, subjects arrived at the hospital 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) on site, the subjects performed a 5-min warm-up exercise at 40% Pmax. After another 10 min of rest, the subjects performed leg-cycle exercise at 110% Pmax until exhaustion. HR was measured continuously during exercise. Time to exhaustion at 110% Pmax (Tex) and HR at exhaustion (HRex) were recorded. A blood sample was collected from the fingertip 3 min after the end of the exercise to determine the blood lactate concentration at exhaustion ([La]ex).

Muscle Fiber, COX Activity, and Microvascular Network Analyses

Cryostat serial transverse sections of 10-μm thick were cut using a microtome at −20°C (HM 560; Microm, Walldorf, Germany).

Immunocytochemical and histochemical assays. Fiber type distribution was studied on immunohistochemical serial preparations using anti-fast IIa myosin heavy chain N2.261 (Alexis Biochemicals) and anti-slow myosin heavy chain A4.951 (Alexis Biochemicals) monoclonal antibodies. The fiber types were designated as I, Ila, and IIX [previously referred to as IIb by Brooke and Kaiser (8)]. Because of the scarcity of hybrid I-IIa and Ila-IIX fibers and for convenience of presentation and statistical analysis, data of type I-IIa and type Ia-IIX fibers were merged with those of type I and IIX, respectively. Microvessels identification and morphometric analysis were performed using the monoclonal antibody CD31 (Dako, Glostrup, Denmark; M0823), which recognizes platelet endothelial cell adhesion molecule-1, a transmembrane glycoprotein strongly expressed by vascular endothelial cells. CD31 successfully permitted identification of vascular endothelium in healthy muscle (10, 15, 16). All slides were incubated at room temperature in an atmosphere saturated with water vapor, for 1 h with primary antibodies (mouse-anti-human), for 30 min with the secondary antibody (rabbit-anti-mouse; Dako P0260), and for 30 min with the tertiary antibody (swine-anti-rabbit; Dako, P0217). The slides were rinsed between incubation with a PBS solution. Peroxidase labeling was performed using a DAB substrate kit (Vector, Burlingame, CA; SK-4100).

Muscle analysis. 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 ×100 magnification for muscle fiber type determination and morphometric analysis [i.e., surface area and perimeter (PF)], quantitative analysis of the microvascular network (i.e., CD), and COX activity (vide infra). The arrangement and morphometric analysis of microvessels, i.e., capillary outer diameter (COD), surface area (CSA), and length of contact between a capillary and a muscle fiber...
Table 1. Characteristics of the subjects and hematological data

<table>
<thead>
<tr>
<th></th>
<th>U-CON (n = 12)</th>
<th>U-SCT (n = 8)</th>
<th>T-CON (n = 10)</th>
<th>T-SCT (n = 9)</th>
<th>PA</th>
<th>HbS</th>
<th>Crossed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric and physiological characteristics</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>23 ± 1</td>
<td>23 ± 1</td>
<td>25 ± 1</td>
<td>23 ± 1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>62 ± 2</td>
<td>67 ± 2*</td>
<td>69 ± 2*</td>
<td>68 ± 2*</td>
<td>0.0334</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HR_{max}, beats/min</td>
<td>182 ± 5</td>
<td>186 ± 5</td>
<td>176 ± 4</td>
<td>187 ± 3</td>
<td>NS</td>
<td>0.0833</td>
<td>NS</td>
</tr>
<tr>
<td>P_{max}, W/kg</td>
<td>2.68 ± 0.09****</td>
<td>2.61 ± 0.11****</td>
<td>3.29 ± 0.09</td>
<td>3.36 ± 0.09</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Hemoglobin and hematological data</strong></td>
<td></td>
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<tr>
<td>HbA1, %</td>
<td>97.1 ± 0.1</td>
<td>58.8 ± 1.0</td>
<td>97.2 ± 0.1</td>
<td>61.3 ± 1.2</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>HbA2, %</td>
<td>2.9 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>2.7 ± 0.2</td>
<td>5.75 ± 0.2</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>HbS, %</td>
<td>n/a</td>
<td>36.0 ± 1.0</td>
<td>n/a</td>
<td>32.9 ± 1.2</td>
<td>NS</td>
<td>n/a</td>
<td>NS</td>
</tr>
<tr>
<td>Hct, %</td>
<td>44.6 ± 0.9</td>
<td>43.8 ± 1.1</td>
<td>42.9 ± 1.2</td>
<td>42.0 ± 1.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>85.6 ± 0.8**</td>
<td>81.6 ± 1.1</td>
<td>84.3 ± 1.3</td>
<td>79.0 ± 0.9</td>
<td>NS</td>
<td>0.0453</td>
<td>NS</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>14.1 ± 0.7</td>
<td>14.2 ± 0.5</td>
<td>13.7 ± 0.3</td>
<td>14.0 ± 0.7</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values presented are means ± SE. U-CON, untrained (Hb)AA subjects; U-SCT, untrained carriers of sickle cell trait (SCT); T-CON, trained HbAA subjects; T-SCT, trained carriers of SCT; PA, effect of regular physical activity; HbS, effect of the SCT carriage; crossed, crossed effect of training and SCT; HR_{max}, maximal heart rate; P_{max}, maximal power; Hct, hematocrit; MCV, mean corpuscular volume; n/a, not applicable; NS, not significant. *P < 0.05, different from U-CON. **P < 0.01, different from T-CON. ***P < 0.001, different from T-SCT. **P < 0.01, different from U-CON. ***P < 0.001, different from T-CON.

Muscle samples (30 mg) were freeze dried (Lyovac GT2; Leybold-Heraeus, Köln, Germany), dissected free from connective and fatty tissues and blood, and powdered in a chamber of controlled humidity (<40% relative humidity). Part of the muscle powder was weighed and homogenized at 4°C in 0.1 M phosphate buffer (pH 8.2) containing 5 mM MgCl₂ and 0.5 mM ATP. This tissue suspension was used to measure fluorometrically at 25°C (SFM25 fluorometer, Kontron Instruments) creatine kinase (CK), phosphofructokinase (PFK), lactate dehydrogenase (LDH), citrate synthase (CS), and β-hydroxyacyl-CoA-dehydrogenase (HAD) activities.

COX activity was obtained by histochemical assessment. Serial transverse sections were incubated for 120 min at 37°C in 0.05 M phosphate buffer (pH 7.3) containing 20 mg 3,3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich), 140 mg cytochrome c (Sigma Biochemical, Poole), 3 g saccharose (Carlo Erba Reactive-SDS), and 4 ml of catalase (Sigma-Aldrich) solution. After incubation, slides were rinsed three times in distilled water and dehydrated in three different alcohol baths. Measurements of COX intensity were performed by converting the image to grayscale to determine optical density for each muscle fiber. The mean relative optical density per pixel was determined by subtracting the optical density of the background. For each subject, a single value per fiber type was obtained by averaging measurements from ~50 well-identified muscular fibers.

**Respiratory Chain Complexes**

The content of the different mitochondrial respiratory chain complexes was studied by western blotting. Approximately 30 mg of muscle were homogenized (Polytron 2100; Kinematica, Newark, NJ) in a sucrose buffer (250 mM sucrose, 30 mM HEPES, 2 mM EDTA, 40 mM NaCl, and 2 mM PMSF, pH 7.4) and centrifuged at 1,000 g for 5 min. This procedure removed heavy material, including a fraction of the mitochondria. The supernatant was spun at 190,000 g for 90 min at 4°C. The new supernatant (cytosolic fraction) was stored at −80°C, while the new pellet (total muscle membrane fraction, including sarcosomal and mitochondrial membrane fractions) was resuspended in Tris-SDS (10 mM Tris, 4% SDS, 1 mM EDTA, and 2

Table 2. Time to exhaustion test findings

<table>
<thead>
<tr>
<th></th>
<th>U-CON (n = 12)</th>
<th>U-SCT (n = 7)</th>
<th>n = 9</th>
<th>T-SCT (n = 8)</th>
<th>PA</th>
<th>HbS</th>
<th>Crossed</th>
</tr>
</thead>
<tbody>
<tr>
<td>110% P_{max}, W</td>
<td>180 ± 6***</td>
<td>194 ± 8***</td>
<td>235 ± 10</td>
<td>237 ± 9</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>T_{ex}, s</td>
<td>169.3 ± 9.4</td>
<td>173.8 ± 7.1</td>
<td>164.2 ± 11.8</td>
<td>164.5 ± 10.9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>W_{ex}, kJ</td>
<td>30.9 ± 1.7*</td>
<td>32.9 ± 2.0†</td>
<td>39.6 ± 3.1</td>
<td>40.6 ± 3.9</td>
<td>0.007</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HR_{ex}, beats/min</td>
<td>187 ± 3</td>
<td>183 ± 5</td>
<td>179 ± 2</td>
<td>182 ± 3</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>[La]_{ex}, mmol/l</td>
<td>9.5 ± 0.3</td>
<td>9.0 ± 0.3</td>
<td>9.3 ± 0.5</td>
<td>9.2 ± 0.6</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values presented are means ± SE. P_{max}, maximal power; T_{ex}, time to exhaustion; W_{ex}, work; HR_{ex}, heart rate at exhaustion; [La]_{ex}, blood lactate concentration ~3 min into recovery after exhaustion. *P < 0.05, different from T-CON. **P < 0.01, different from T-CON. ***P < 0.001, different from T-SCT. $P < 0.05$ and **$P < 0.01$, different from T-SCT. Other symbols: see Table 1.
mM PMSF, pH 7.4). The protein content was determined with a BSA standard (DC protein assay; Bio-Rad, Marnes-la-Coquette, France). Both two and four micrograms of protein from each sample were electrophoresed on a 15% SDS polyacrylamide gel for 3 h (150 V at +4°C) with a Mini-Protein II system (Bio-Rad) in a Tris-glycine-SDS buffer. Then, proteins were transferred onto nitrocellulose membranes during 2 h (150 mA at +4°C) in a Tris-glycine buffer with 20% methanol. Membranes were blocked 3 h with a buffer containing PBS-Tween (0.1%), and 5% no-fat milk. The primary antibody cocktail against respiratory chain subunits (MitoProfile Total OXPHOS Human WB Antibody Cocktail, ref. MS601; Mitosciences, Paris, France) was diluted (1:400) in a PBS-Tween (0.1%) buffer with 1% low fat milk. Horseradish peroxidase-conjugated goat anti-mouse IgG at a 1:10,000 dilution was used as a secondary antibody. Detection of membrane bound proteins was performed using an ECL chemiluminescence kit (Amersham, Courtaboeuf, France) and visualized on a hyper film (Amersham). Then, the different respiratory complexes were scanned with a densitometer system equipped with an integrator (GS-800; Bio-Rad, Marne-la-Coquette, France) and quantified using the Quantity-One software (Bio-Rad).

Statistical Analysis

Descriptive statistics are expressed as means ± SE. Because α-thalassemia may constitute an important confounding factor, analysis of covariance was performed using α-thalassemia as a covariate for each parameters tested. Except for hematological parameters, α-thalassemia did have any effect on the studied muscular parameters. Subsequently, two-way ANOVA procedures were used to determine the effects of SCT, endurance training, and the interaction of these two factors on the various parameters of interest. Pair-wise comparisons (Fisher least significant difference post hoc tests) were used when necessary to locate where significant differences had occurred. Cross effects were studied by means of linear regressions. 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

While age was not different among the four groups, Table 1 shows that body mass and Pmax (W/kg) were significantly higher in the trained groups. Pmax was higher by 23% and 29% in T-CON and T-SCT, respectively, than untrained counterparts. Besides, HRmax tended to be higher in the SCT groups.

| Hemoglobin and Hematological Data |

The percentage of HbA1 and HbA2 were higher in the control groups compared with the SCT groups (Table 1). Mean corpuscular volume was significantly lower in carriers of SCT. There were no differences among the groups for Hct and Hb content. The percentage of Hbs was similar between the two SCT subgroups (i.e., U- and T-SCT).

Time to Exhastuation at 110% P max

Some subjects did not reach HR max at the end of the Tex, suggesting they stopped exercise before exhaustion. Besides, some blood samples could not be taken on time at the end of Tex. In those cases, the validity of HRex, Tex and/or [La]ex was doubtful. Consequently, the values of three subjects have been discarded (see the n value in Table 2). Power output (i.e., 110% P max) and mechanical work (Wex) performed during Tex were higher in the trained than in the untrained groups but were similar between the subgroups, i.e., CON and SCT (Table 2). Wex was higher by 13 and 12% in T-CON and T-SCT, respectively than untrained counterparts. There were no differences among the different groups neither for Tex, HRex, nor [La]ex.

| Table 4. Muscle enzyme activities |

Values are means ± SE and are expressed for all activities as µmol·min⁻¹·g⁻¹ dry muscle excepted for cytochrome c oxidase activity (COX; for each fiber type), which are expressed in relative optical density. CK, creatine kinase; PFK, phospho-fructo kinase; LDH, lactate dehydrogenase; CS, citrate synthase; HAD, β-hydroxyl acyl Co-A dehydrogenase. *P < 0.05, different from U-CON. $P < 0.05, different from T-SCT. **P < 0.01, different from T-CON. Other symbols: see Table 1.
Table 5. Content of the different mitochondrial respiratory chain complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>U-CON (n = 9)</th>
<th>U-SCT (n = 6)</th>
<th>T-CON (n = 10)</th>
<th>T-SCT (n = 8)</th>
<th>PA</th>
<th>HbS</th>
<th>Crossed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>0.85 ± 0.04</td>
<td>0.79 ± 0.08</td>
<td>1.01 ± 0.07</td>
<td>1.02 ± 0.06</td>
<td>0.0088</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Complex II</td>
<td>0.76 ± 0.06</td>
<td>1.04 ± 0.22</td>
<td>1.01 ± 0.12</td>
<td>0.97 ± 0.15</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Complex III</td>
<td>0.66 ± 0.06</td>
<td>0.85 ± 0.13</td>
<td>1.14 ± 0.12**</td>
<td>0.79 ± 0.05</td>
<td>0.0344</td>
<td>NS</td>
<td>0.0120</td>
</tr>
<tr>
<td>Complex IV</td>
<td>0.56 ± 0.05</td>
<td>0.66 ± 0.12</td>
<td>1.09 ± 0.08**</td>
<td>1.02 ± 0.12*</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Complex V</td>
<td>0.87 ± 0.08</td>
<td>0.99 ± 0.20</td>
<td>1.18 ± 0.11</td>
<td>1.05 ± 0.17</td>
<td>0.0992</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE and are expressed as arbitrary units. *P < 0.05, different from U-CON, **P < 0.01, different from U-CON, †P < 0.05, different from U-SCT. Other symbols: see Table 1.

Muscle Fiber Type Distribution and Surface Area

Muscle fiber type distribution was 33.1 ± 1.9, 51.7 ± 1.9, and 15.2 ± 1.9% for type I, IIA, and IIX, respectively. No fiber type distribution intergroup differences were detected (Table 3). Nevertheless, there was a trend toward lower type I fibers percentage in the trained carriers of SCT compared with the trained control group.

Training was associated with higher surface area of type II fibers in both CON and SCT subjects. Statistical analysis demonstrated that there were trends toward higher mean surface area of type I and IIX fibers in the SCT groups compared with the two control groups (Table 3). Untrained carriers of SCT had significantly higher type I and IIX fibers surface area than untrained control subjects. While T-CON displayed significant higher type IIa surface area than U-CON, T-SCT showed similar (not significant lower) type I and IIX fiber surface areas than their untrained counterparts (U-SCT).

Enzyme Activities

The skeletal muscle key enzyme activities are shown in Table 4. The effect of training on CK activity is divergent between CON and SCT. While training status was associated with a higher CK activity in CON, CK activity was lower in T-SCT than in U-SCT (Table 4). Consequently, T-SCT CK activity was lower than the three other groups. No significant differences among the groups emerged for PFK, LDH, CS, and HAD. Statistical analysis showed a trend toward a higher HAD activity in T-CON compared with U-CON. Trained subjects tended to have higher CKO enzyme activity in all fiber types compared with untrained subjects. Besides, we observed trends toward lower COX activity in all fiber types of carriers of SCT (U- and T-SCT) compared with the control groups (Table 4). Differences reached statistical significance for the untrained carriers of SCT compared with the untrained control group for types I and IIA fibers.

Mitochondrial Respiratory Chain Complexes

Regular physical activity was associated with higher contents in electron transport chain complexes I, III, IV, and V. (Table 5). However, trained carriers of SCT showed unchanged expression of complexes II, III, and V compared with U-SCT.

Microvascular Network

Some of the microvascular network characteristics are shown in Table 6 and Figs. 1 and 2. Regular physical activity was associated with improved microvascular parameters (CD, CAF, LC/PF, COD, CapTor, CSA, CD × CPm, and CD × CSA). CD was lower in carriers of SCT (P = 0.0026; Fig. 1). CD was significantly higher in the T-CON group compared with the three other groups. T-SCT subjects had similar CD to U-CON (Fig. 1). Carriers of SCT displayed lower CapTor and trends toward lower CAF, LC/PF and CSA. Carriers of SCT were also associated with significantly higher COD. Of note, CapTor does not respond to physical activity in carriers of SCT (Fig. 2).

DISCUSSION

The aim of this study was to determine whether regular physical activity induces muscle adaptations in carriers of SCT as it occurs in normal HbAA (CON) subjects. One main finding of the present study is that carriers of SCT adapted almost similarly to CON to regular physical activity for most (but not all, see below) of the functional and structural muscle characteristics. Of note, these proportionally similar adaptations lead to the fact that the differences observed between U-SCT and U-CON remain in the active groups of subjects.

Table 6. Microvascular characteristics

<table>
<thead>
<tr>
<th>U-CON (n = 12)</th>
<th>U-SCT (n = 8)</th>
<th>T-CON (n = 10)</th>
<th>T-SCT (n = 9)</th>
<th>PA</th>
<th>HbS</th>
<th>Crossed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD, cap/mm²</td>
<td>308.4 ± 6.8*</td>
<td>266.9 ± 5.8**</td>
<td>383.2 ± 12.9</td>
<td>324.5 ± 8.1*</td>
<td>0.0001</td>
<td>0.0026</td>
</tr>
<tr>
<td>CAF, mean, cap</td>
<td>4.65 ± 0.4†</td>
<td>4.08 ± 0.22*</td>
<td>5.62 ± 0.49</td>
<td>4.81 ± 0.27</td>
<td>0.0386</td>
<td>0.0881</td>
</tr>
<tr>
<td>LC/PF, mean, %</td>
<td>15.3 ± 1.0***</td>
<td>14.3 ± 1.1***</td>
<td>21.3 ± 0.8</td>
<td>19.4 ± 1.6</td>
<td>0.0001</td>
<td>0.0778</td>
</tr>
<tr>
<td>COD, μm</td>
<td>5.18 ± 0.15***</td>
<td>5.79 ± 0.9***</td>
<td>6.11 ± 0.31*</td>
<td>6.90 ± 0.26</td>
<td>0.0001</td>
<td>0.0049</td>
</tr>
<tr>
<td>Cap, Tor</td>
<td>5.21 ± 0.31***</td>
<td>4.85 ± 0.27**</td>
<td>6.29 ± 0.23***</td>
<td>4.79 ± 0.25</td>
<td>0.0437</td>
<td>0.0006</td>
</tr>
<tr>
<td>CSA, μm²</td>
<td>114.7 ± 3.2***</td>
<td>132.2 ± 5.9***</td>
<td>175.2 ± 8.6</td>
<td>191.7 ± 14.5</td>
<td>&lt;0.0001</td>
<td>0.0651</td>
</tr>
<tr>
<td>CD × CP, (mean, mm)</td>
<td>7.21 ± 0.41</td>
<td>6.95 ± 0.39</td>
<td>12.05 ± 1.10</td>
<td>10.86 ± 0.64</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>CD × CSA, μm²/mm²</td>
<td>20.6 ± 0.7</td>
<td>20.8 ± 1.4</td>
<td>39.9 ± 3.4</td>
<td>36.7 ± 3.3</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. CD, capillary density; CAF, number of capillaries around single fiber (cap); LC/PF: length of contact between capillaries and the perimeter of the fiber; COD, capillary outer diameter; CapTor, capillary tortuosity; CSA, capillary surface area; CP, capillary perimeter. *P < 0.05, different from T-CON. **P < 0.01, different from T-CON. ***P < 0.001, different from T-CON with. †P < 0.05, different from T-SCT. ††P < 0.01, different from T-SCT. $P < 0.01, different from T-SCT. Crossed: see Table 1.
Specifically, CD, CAF, LC/PF, and COX remained or tended to remain lower, and COD and CSA remained or tended to remain higher in T-SCT than in T-CON. Another important finding is that SCT subjects displayed some specific muscular adaptations with regular physical activity. Indeed, CK activity was lower while complexes II, III, and V content and capillary tortuosity remained unaltered in active compared with untrained carriers of SCT.

**General Comments on the Effect of Physical Activity**

In control (HbAA) subjects, regular physical activity was accompanied by higher maximal aerobic power and work during time to exhaustion test (Table 2), COX activities in all fiber types (I, IIa, and IIX; Table 4), content of most of the mitochondrial respiratory chain complexes (Table 5), CD (Fig. 1), number of capillaries around fiber, length of contact between capillaries and muscle fiber and capillary tortuosity, diameter, and surface area (Table 6). Besides, even if they did not reach statistical threshold, our results tended to show an increase in CS activity in trained subjects (+16% between U- and T-CON; Table 7). The failure to reach statistical significance for CS activity can be explained by the conjunction of two factors, i.e., 1) the studied populations, and 2) the cross-sectional design of the study. Because our trained subjects were collegiate soccer players and not highly trained endurance athletes, the difference between T-CON and U-CON (untrained young adults) is relatively narrow and somewhat shaded by the cross-sectional design of the study.

**Table 7. Synthesis of changes with training**

<table>
<thead>
<tr>
<th></th>
<th>T-CON vs. U-CON, %</th>
<th>T-SCT vs. U-SCT, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;max&lt;/sub&gt;, W/kg</td>
<td>+23</td>
<td>+29</td>
</tr>
<tr>
<td>W&lt;sub&gt;ex&lt;/sub&gt;, kJ</td>
<td>+13</td>
<td>+12</td>
</tr>
<tr>
<td>CS</td>
<td>+16</td>
<td>+10</td>
</tr>
<tr>
<td>COX type I</td>
<td>+10</td>
<td>+10</td>
</tr>
<tr>
<td>COX type IIa</td>
<td>+9</td>
<td>+7</td>
</tr>
<tr>
<td>RC complex I</td>
<td>+20</td>
<td>+28</td>
</tr>
<tr>
<td>RC complex II</td>
<td>+33</td>
<td>-6</td>
</tr>
<tr>
<td>RC complex III</td>
<td>+71</td>
<td>-7</td>
</tr>
<tr>
<td>RC complex IV</td>
<td>+93</td>
<td>+12</td>
</tr>
<tr>
<td>RC complex V</td>
<td>+34</td>
<td>+6</td>
</tr>
<tr>
<td>CD, cap/mm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>+24</td>
<td>+22</td>
</tr>
<tr>
<td>CAF, mean, cap</td>
<td>+21</td>
<td>+18</td>
</tr>
<tr>
<td>CapTor</td>
<td>+17</td>
<td>-1.2</td>
</tr>
</tbody>
</table>

RC, respiratory chain.

**Effect of Regular Physical Activity in Carriers of SCT**

In agreement with our original hypothesis, the present study showed that carriers of SCT adapted to regular physical activity nearly similarly to their control counterparts. Indeed, almost all indexes of physical ability as well as functional and structural skeletal muscle characteristics were higher in physically active carriers of SCT. Furthermore, these improvements were proportionally similar to those reported in HbAA counterparts for most of the studies’ parameters (Table 7).

Of note, the similarity in the effects of physical activity implies that the differences observed between SCT and CON subjects in the sedentary state remain in the active state. This is the case for CD, CAF, LC/PF, and COX, which remain lower, and for COD and CSA, which remain higher in T-SCT than in T-CON. In the present study, CD and COX activity of T-SCT are of the same order of magnitude as those observed in the sedentary control subjects (U-CON).

On the other hand, some specific adaptations associated with regular physical activity were found in our T-SCT group. Indeed, CK activity; respiratory chain complexes II, III, and V; and type I and IIX fiber surface areas and capillary tortuosity were similar or even lower in the active carriers of SCT (T-SCT) than in their sedentary counterparts (U-SCT), i.e., rather than an improvement, regular physical activity did not change or even depressed some functional and structural muscle characteristics in the carriers of SCT.

Since CK plays also a key role in the regulation of mitochondrial respiration as an energetic shuttle in the cell (49, 50, 57), the lower CK activity in the physically active carriers of SCT (T-SCT) than in the three other groups may indicate an impaired oxidative metabolism in the former subjects. This hypothesis is reinforced by the deleterious effect of HbS on COX activity in all fiber types (Table 4), as well as by the lack of effect of regular physical activity on the content of complexes II, III, and V of the mitochondrial respiratory chain in the T-SCT subjects, contrary to the observed in T-CON (Table 5). The altered oxidative potential observed in the present study in the T-SCT might constitute a possible explanation for the relatively modest performances observed in endurance-trained SCT athletes for half-marathon (38). Because previous works showed that reactive oxygen species lead to inhibition of both CK (23, 35, 36) and COX (15, 25, 52), it is therefore possible that the lower CK and COX activities in T-SCT...
subjects might be related to repeated muscle oxidative stress in response to regular exercise (i.e., training) in carriers of SCT. The lack of difference in CK between U-CON and U-SCT would further argue in favor of the deleterious effects of muscle reactive oxygen species production associated with regular exercise on CK activity. However, further studies are needed to test specifically this hypothesis.

Already lower in U-SCT than in U-CON, CapTor remained unchanged in active carriers of SCT (T-SCT, see Fig. 2). CD remained also lower in T-SCT than in T-CON. On the other hand, COD, already higher in U-SCT than in U-CON, remained higher in T-SCT than in T-CON. In other words, the particular remodeling of the skeletal muscle microvasculature observed in U-SCT still persists and is even amplified in T-SCT. The exact mechanisms underlying this particular remodeling of the skeletal muscle microvasculature are not known yet. In a previous work (55), we postulated that the particular microvascular remodeling already observed in U-SCT subjects 1) might be related to their hemorheological disorders (lower RBC deformability, higher apparent blood viscosity, and increased vascular adhesion), and 2) might allow to dampen the possible risks related to their hemorheological disorders while maintaining a similar local blood flow to HbAA subjects. These inferences were deduced from the following assumptions and results: first, because HbS-containing RBCs are slightly less deformable and could adhere more to the endothelium than normal RBCs (13, 43), the lower microvascular network anisotropy (assessed in the present study by CapTor) would reduce both flow resistance in the microcirculation and RBC transit time in the capillaries. Thus a reduced CapTor would dampen the risk for blood flow disorders and attendant deleterious consequences, i.e., sickling and microvascular occlusion. Second, the enlargement of the microvessels seems to compensate for the lower CD and tortuosity. Indeed, the product CD × CSA that denotes microvascular blood supply, albeit very imperfectly (29), was different in carriers of SCT compared with HbAA subjects (55). The results we report here in trained carriers of SCT show that the adaptations reported in less active carriers of SCT are amplified in the trained state. In other words, these adaptations, i.e., unchanged CapTor and higher COD, may further dampen the hemorheological disturbances encountered by carriers of SCT during exercise. In accordance with this hypothesis, Aufradet et al. (3) reported recently that a physically active lifestyle might decrease endothelial activation in carriers of SCT.

Summary

Functional and structural skeletal muscle characteristics of carriers of SCT adapted almost all similarly to HbAA subjects with regular physical activity. However, because 1) some parameters were different in sedentary SCT than in their CON counterparts, and 2) the changes in response to regular physical activity were proportionally similar in SCT and CON groups, these parameters remained different in T-SCT and T-CON. Specifically, CD, CAF, LC/PF, and COX activity remained lower, while COD and CSA remained higher in T-SCT than in T-CON. In addition to COX activity, the results of CK activity and content of complexes II, III, and V suggest that oxidative potential is altered in physically active carriers of SCT compared with HbAA counterparts. The specific remodeling of the skeletal muscle microvascular network may be the consequence of adaptive mechanisms against the deleterious presence of HbS while an adequate local blood flow is maintained.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


