The American Journal of Pathology, Vol. 185, No. 5, May 2015

ELSEVIER

The American Journal of
PATHOLOGY
ajp.amjpathol.org

MUSCULOSKELETAL PATHOLOGY

Evidence for a Profound Remodeling of Skeletal Muscle and Its Microvasculature in Sickle Cell Anemia

Marion Ravelojaona,* Léonard Féasson,*† Samuel Oyono-Enguéllé,‡ Lucile Vincent,§ Ben Djoubairou,‡ Charles Ewa'Sama Essoue,‡ and Laurent A. Messonnier§

From the Laboratory of Exercise Physiology EA4338, University of Lyon, Saint Etienne; the Myology Unit — Rhônes-Alpes Reference Center for Rare Neuromuscular Diseases — CHU of Saint Etienne,† Saint Etienne, France; the Laboratory of Physiology,‡ Medical School and Biomedical Sciences Faculty, University of Yaounde I, Yaoundé, Cameroon; and the Laboratory of Exercise Physiology EA4338,§ Savoie Mont Blanc University, Chambéry, France*

Accepted for publication January 23, 2015.

Address correspondence to Laurent A. Messonnier, Ph.D., Laboratoire de Physiologie de l'Exercice EA4338, Université Savoie Mont Blanc, F-73376 Le Bourget-du-Lac Cedex, France. E-mail: laurent.messonnier@univ-savoie.fr.

Sickle cell anemia (SCA) is a hemoglobinopathy leading to major hematologic, hemorheologic, and hemodynamic disorders that induce various complications, including organ failure, and ultimately lead to death. Here, we assessed for the first time repercussions of SCA on skeletal muscle and its microvasculature. Twenty-seven sedentary Cameroonian volunteer men participated in the study. They were assigned to one of three groups according to their hemoglobin status (healthy control subjects, $n = 10$; sickle cell trait carriers, $n = 10$; and SCA patients, $n = 7$) and underwent muscle biopsy of the *vastus lateralis*. SCA was associated with microvessel rarefaction, decrease in capillary tortuosity, and widening of microvessel diameter. The absence of capillary wall reinforcement was shown by lack of wall thickening and lack of fibrous tissue or smooth muscle in their constitution. We also observed changes in fiber type distribution, muscle atrophy, an increase in satellite cell number, and a decrease in activity of creatine kinase and several oxidative enzymes. No signs of tissue necrosis, inflammatory stress, fibrosis, or segmented fibers were observed. The present study highlighted marked effects of SCA on microvascular, structural, and energetic characteristics of skeletal muscle. *(Am J Pathol 2015, 185: 1448—1456; http://dx.doi.org/10.1016/j.ajpath.2015.01.023)*

Sickle cell anemia (SCA) is a genetic hemoglobinopathy leading to synthesis of abnormal hemoglobin (Hb)S. In its deoxygenated form, the mutated hemoglobin polymerizes, giving the red blood cells their particular sickle shape. Because hemolysis related to the fragility of the sickled erythrocytes is not compensated by erythropoiesis, the first clinical manifestation of the disease is severe hemolytic anemia. Sickled red blood cells also display a loss of deformability and an abnormal adhesion to the endothelium that favor the entrapment of erythrocytes in the microvasculature, resulting in vaso-occlusive crises[1] and infarction of vital organs. Resolution of vaso-occlusive episodes amplifies inflammation[2–5] and oxidative stress[4,6] that contribute to the pathophysiology of SCA and especially vasculopathy. These vaso-occlusive or vasculopathic phenotypes often result in failure of critical organs such as the spleen, kidneys, liver, brain, lungs, and bones.[7]

Literature related to skeletal muscle in SCA is relatively sparse, maybe because of its less vital nature, but also because sickle myonecrosis episodes are relatively rare complications.[8–12] However, richly microvascularized and sensitive to hypoxia and anoxia, oxidative stress, and inflammation, skeletal muscle may sustain severe damage from the disease. Occlusion and ischemia-reperfusion episodes are known to induce profound microvascular functional and structural remodeling, including alterations of capillary perfusion[4] (no reflow phenomena[3,5]) and a

Supported by grants from the Embassy of France in Cameroon (L.F., L.V. and L.M.), the Région Rhône-Alpes (M.R., L.F., L.V. and L.M.), and the Ministry of Higher Education of Cameroon (S.O.-E.).

M.R. and L.F. contributed equally to this work.

Disclosures: None declared.

http://dx.doi.org/10.1016/j.ajpath.2015.01.023

Table 1 Anthropometric, Hemoglobinic, and Hematologic Data of the Participants

Characteristic	CON, $n = 10$	SCT, $n = 10$	SCA, $n = 7$
Anthropometric data			
Age, years	25 ± 1	23 ± 1	24 ± 2
Height, cm	176 ± 2	176 ± 1	171 ± 3
Body mass, kg	72 ± 2	68 ± 2	59 ± 3***†
Body fat, %	13 ± 1	11 ± 1	11 ± 1
Body mass index, kg/m^2	23.1 ± 0.6	21.9 ± 0.4	20.6 ± 0.8**
Hemoglobinic and hematological data			
HbS, %		31.65 ± 1.89***	80.90 ± 2.02***†††
HbF, %		0.08 ± 0.08	4.63 ± 1.36***†††
Hb, g/dL	14.1 ± 0.3	13.4 ± 0.3	8.8 ± 0.6***†††
Hct, %	41.1 ± 0.8	39.8 ± 0.6	25.2 ± 1.9***†††

Data are expressed as means ± SEM.
**$P < 0.01$, ***$P < 0.001$ versus CON.
†$P < 0.05$, †††$P < 0.001$ versus SCT.
CON, healthy control; Hb, hemoglobin total; HbF, fetal hemoglobin; HbS, hemoglobin S; Hct, hematocrit; SCA, sickle cell anemia; SCT, sickle cell trait.

decrease in capillary density.[13] Hypoxemia related to anemia and arterial oxyhemoglobin desaturation[14,15] may induce chronic periods of tissular hypoxia, which has been reported ultimately to depress muscle oxidative capacity via tumor necrosis factor-α and NF-κB cascades.[16,17] Furthermore, reperfusion subsequent to ischemia also is known to induce alteration of muscle oxidative phenotype.[16] From these points of view, changes in muscle energetics can be assumed in patients with SCA. Moreover, ischemia and tissular anoxia related to vaso-occlusive crises potentially may lead to tissue necrosis,[3] and the pro-inflammatory state[2–5] and oxidative stress[4,6] related to reperfusion subsequent to ischemia also are known to induce muscle atrophy[18,19] and possibly necrosis.[3,20] Therefore, even if myonecrosis is not a leading component of sickle crisis,[11] the major hematologic, hemorheologic, and hemodynamic disorders associated with SCA could lead to a profound remodeling of skeletal muscle and its microvasculature, which could worsen patients' morbidity and loss of autonomy. Because of the lack of knowledge of muscular repercussions of SCA, we studied microvascular, structural, anatomopathological, and energetic characteristics of skeletal muscle in homozygous patients, heterozygous carriers, and control subjects.

Materials and Methods

Participants

Twenty-seven sedentary Cameroonian men [healthy control (CON) subjects, $n = 10$; sickle cell trait (SCT) carriers, $n = 10$; and SCA patients, $n = 7$] participated in this study (Table 1), which took place at the Yaoundé General Hospital (Cameroon). The experiment was approved by the ethics committee (no. 02–06–2007) and conformed to the standards set by the Declaration of Helsinki for human studies. Before giving their written consent, eligible volunteers were informed fully of the objectives, procedures, and possible risks and discomforts related to the protocol.

Inclusion Criteria

All subjects underwent preliminary screening, including a physical examination and a blood phenotype analysis (Table 1). Volunteers who i) had had a malaria episode within the previous two months; ii) had more than three vaso-occlusive crises per year that required hospitalization ie, displayed a severe clinical phenotype[21]; iii) took any medications; iv) tested positive for HIV; or v) were taking part in another research program were not included in the study.

Muscle Biopsy

Subjects arrived at the hospital at either 8 AM or noon. Muscle biopsy of the right *vastus lateralis* was performed percutaneously.[22] After the patient was shaved, asepsis was attained using alcohol and iso-Betadine 10% (MEDA Pharma, Paris, France), and local anesthesia of cutaneous and subcutaneous tissues was attained (2% lidocaine; AstraZeneca, Rueil-Malmaison, France), without crossing the muscular aponeurosis. A small incision was made until the crossing of the epimysium, through which a Weil-Blakesley forceps (Lawton, Tuttlingen, Germany) was introduced and the sample extracted (approximately 100 to 150 mg). Part of the biopsy sample was mounted in Cryomount (Histolab, Göteborg, Sweden), then frozen in isopentane (Chevron Phillips Chemicals International, Overijse, Belgium), and finally stored in liquid nitrogen until histochemical and immunohistochemical analyses were performed on 10-μm thick serial cryostat transverse sections. The remainder of the sample (frozen and stored in liquid nitrogen) was devoted to enzyme activity analyses.

Microvascular Network Analysis

Morphometric analysis of microvasculature was performed as done previously.[23] Briefly, capillaries were identified with CD31 antibody (Dako, Glostrup, Denmark), which recognizes platelet endothelial cell adhesion molecule 1, expressed by vascular endothelial cells. Capillary wall thickness was measured with