

## Resting blood lactate in individuals with sickle cell disease

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**Background:** The most common hereditary hemoglobin disorder, affecting 20 million individuals worldwide, is sickle cell disease. The vascular obstruction resulting from the sickling of cells in this disease can produce local hypoxemia, pain crises and infarction in several tissues, including the bones, spleen, kidneys and lungs.

**Objective:** To determine red blood group genes in a Brazilian populations.

**Methods:** The present study is characterized as a case control study, with the aim of identifying the baseline blood lactate concentration in individuals with hemoglobin SS and SC diseases. One-way ANOVA with the Tukey post-test was used to analyze the results and a p-value < 0.05 was considered significant. Calculations were made using the INSTAT statistical program. The graphs were generated using the ORING program. The study sample was composed of 31 men and women residing in the city of Santo Antônio de Jesus, Bahia, Brazil. The individuals were divided into two groups: Group GC of 16 subjects who did not present with any type of structural hemoglobinopathy; and Group GE composed of 15 individuals with ages between 2 and 35 years old, who had the SS and SC genotypes. Sample analyses were performed with 3 mL of blood during fasting.

**Results:** The baseline blood lactate concentration of the SS and SC individuals was higher than that of the control group ( $p < 0.001$ ) with means of  $4.86 \pm 0.95$ ;  $3.30 \pm 0.33$ ;  $1.31 \pm 0.08$  IU/L for SS, SC and controls, respectively. This corroborates the initial research hypothesis.

**Conclusion:** The baseline blood lactate of SS and SC individuals is 3 to 4 times higher than that of healthy subjects, probably due to the fact that these patients have a metabolic deviation to the anaerobic pathway.

**Keywords:** Sickle cell anemia; Lactic acid; Physical exercise

### Introduction

Hereditary hemoglobin disorders are the most common genetic diseases of humankind.<sup>(1)</sup> The most important of these is sickle cell disease, a disorder that affects 20 million people worldwide.<sup>(2)</sup> Few populations are exempt and its presence in many regions is a concern for public health.<sup>(3,4)</sup> Therefore, considering the heterogeneous origin of the Brazilian population, it is not surprising that hereditary hemoglobin disorders are common and that their occurrence varies in different regions.<sup>(3)</sup>

Sickle cell disease is characterized by a point mutation (GAG-GTG), corresponding to the substitution of glutamic acid by valine at position 6 of the beta chain of the hemoglobin molecule, giving rise to the hemoglobin mutant S (Hb S).<sup>(5-7)</sup> This small alteration changes the entire rheology of the red blood cell; the primary process of this event is polymerization or jellification of hemoglobin.<sup>(8,9)</sup> The speed and extent of the formation of polymers within red blood cells primarily depend on three factors: The degree of deoxygenating, intracellular concentration of hemoglobin S (Hb S) and presence or absence of Fetal Hemoglobin (Hb F).<sup>(10)</sup>

This change in the amino acid alters the characteristics of the hemoglobin molecule in such a manner that red blood cells become sickle-shaped under conditions of low oxygen tension, making them less flexible and unable to move easily. The resultant vascular obstruction produces local hypoxemia, pain crises and infarction in various tissues, including the bones, spleen, kidneys and lungs. The premature destruction of sickled cells diminishes the level of hemoglobin, causing anemia.<sup>(1)</sup> These factors, associated with sickled cell adherence to the endothelium, favor the formation of thrombi in the micro and macrocirculation.<sup>(10,11)</sup>

The main signs and symptoms of sickle cell disease are: greater susceptibility to infections; osteoarticular manifestations, such as aseptic femur head necrosis and hand-foot syndrome, splenomegaly, hepatomegaly, strokes, priapism, leg ulcers, pain crises, fever, pulmonary complications,<sup>(12,13)</sup> and alterations in heart function. Heart alterations

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involve cardiomegaly, systolic heart murmur, electrocardiographic alterations (alterations of the T wave or ST segment), uni- or bilateral hypertrophy (with hypertrophy of the right ventricle appearing later and being less intense) and increases in pre-load and reductions in post-load.<sup>(14-17)</sup> Cardiovascular disease is a frequent clinical manifestation in sickle cell disease and contributes to the increases in morbidity and mortality of these individuals.<sup>(17)</sup>

The general treatment is based on providing clinical support and specific care<sup>(2,18)</sup> with the goal of minimizing and preventing the consequences of chronic anemia, sickling crises and susceptibility to infections.<sup>(8)</sup> At present, a curative treatment, by hematopoietic stem cell transplant is being studied.<sup>(19)</sup>

It is worth pointing out that there is no indication for sickle cell anemia individuals to perform intense physical exercise, as this increases the demand for oxygen, overloading the red blood cells that may under these circumstances become sickled, and consequently, occlude blood vessels, resulting in vaso-occlusive crises.<sup>(20-22)</sup> Therefore, a different type of physical exercise program is suggested in order to avoid such crises. It is important to determine at what time the lactate threshold occurs in these individuals, as their baseline blood lactate level may be higher than in healthy individuals.

The lactate threshold is defined as the point during exercise, at which the serum lactate rapidly begins to accumulate above the baseline concentration.<sup>(23)</sup> The onset of this accumulation indicates an imbalance between the production and removal of blood lactate denominated as the anaerobiosis threshold (AT). The standard value, fixed at 4 mmol of lactate per liter of blood, is used as a common point of reference.<sup>(23-27)</sup>

It is believed that exercise leads to increases in the oxidation of lactic acid by muscles and more intense exercises could result in an increase in the production of lactic acid and prevent it from being removed. Therefore, the increase in the blood lactic acid concentration may occur as a result of both its production and the reduction in its removal.<sup>(25)</sup>

Therefore the aim of the present study is to measure the baseline blood lactate levels in sickle cell disease individuals with the purpose of seeing whether there is a difference between the SS and SC genotypes in respect to lactate and compare this with the level found in healthy individuals.

## Methods

The present study is a case control study, in which 31 individuals were divided into two groups, Control (CG) and Experimental (EG).

The EG consisted of 15 volunteer men and women aged between 2 and 35 years old with laboratory diagnosis of sickle cell disease, who were regularly attended at the Hematology

Clinic in the city of Santo Antônio de Jesus, BA, Brazil. The EG volunteers were further subdivided into two sub-groups, six individuals with the SS genotype and a mean age of 18 years old and nine individuals with the SC genotype and a mean age of 13 years old.

The laboratory diagnoses of sickle cell disease and genotypes (SS or SC) were obtained by the association of alkaline (cellulose acetate) and acid (agar) electrophoresis techniques complemented by the Hb F concentration. Hb F was measured by the alkaline denaturing method based on its higher resistance compared to other hemoglobins.<sup>(26)</sup> These diagnostic techniques are considered the most precise to detect sickle cell disease and the respective genotypes.<sup>(27,28)</sup>

The CG consisted of 16 male and female volunteers with a mean age of 17 years old but without any type of structural hemoglobinopathy.

Although it is known that age has a direct influence on the clinical symptoms and manifestations of sickle cell disease,<sup>(9)</sup> no age range was delimited in this study as the volunteers in the EG and CG Groups did not presented any hepatic or renal involvement that could have led to inadequate metabolism of blood lactate and consequently that could have influenced the results of the study.

### *Ethical aspects*

After having received guidance about their participation in the research, and information about the risks and benefits of the research, the volunteers signed an informed consent form in accordance with Resolution CNS 196/1996 related to the Guidelines and Rules Regulating Research Involving Human Beings. In cases of under 18-year-old volunteers, the parents or guardians signed the consent form. This research was registered with and approved by the Research Ethics Committee of the Adventist Physiotherapy School under CAAE No. 0032.0.070.000-05.

### *Collection procedure*

After 12h fasting, 3 mL of blood was collected from each of the 31 participants to measure the baseline lactate level. To measure blood lactate, the serum was separated from the plasma by centrifugation and harvested. Subsequently the serum was separated and the spectrophotometric method was used to quantify blood lactate.

### *Statistical analysis*

Data were evaluated by descriptive analysis for all the variables. The variables are expressed as means  $\pm$  standard error. One-way ANOVA complemented by the Tukey-Kramer post-test was used to analyze the results and identify possible significant differences. The level of significance adopted was for p-values  $< 0.05$  with a 95% confidence interval. The calculations were made using the GraphPad InStat 2.01 statistics program. Graphs were generated using the ORING program.

Table 1 - Hematologic characterization of the study sample (Mean ± standard deviation)

	Hematocrit (%)	Hemoglobin (g/dL)	RBC (10 <sup>6</sup> /mm)	Leukocytes (10 <sup>3</sup> /mm)	Band neutrophils (%)	Segmented neutrophils (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
Control	38.5± 0.9	12.75 ± 0.7	4.3 ± 0.1	6.5 ± 342.3	1.8 ± 0.3	57 ± 1.3	1.4 ± 0.1	37.5 ± 2.2	1.4 ± 0.1
SS	24 ± 2	6.95 ± 0.6	3.28 ± 0.2	10.63 ± 1586.6	1.17 ± 0.2	48.17 ± 5.6	3.5 ± 0.4	46.0 ± 5.6	1.17 ± 0.2
SC	32.55 ± 1.1	9.68 ± 1.1	3.96 ± 0.1	9.26 ± 692.6	1.66 ± 0.5	53.11 ± 2.4	5.11 ± 1.3	38.44 ± 3.8	1.66 ± 0.3

## Results

The hematologic data of the study sample are shown in Table 1.

The data show that the baseline blood lactate concentration for Hb SS and Hb SC individuals was three to four times higher than that of the Control Group ( $p < 0.001$ ), with mean values of  $4.86 \pm 0.95$ ;  $3.30 \pm 0.33$ ;  $1.31 \pm 0.08$  IU/L, respectively. A significant difference ( $p < 0.05$ ) was observed between the baseline blood lactate of Hb SS individuals compared to Hb SC individuals with the value for Hb SS being higher than that for Hb SC, which possibly justifies the more severe clinical conditions seen in these individuals (Figure 1).

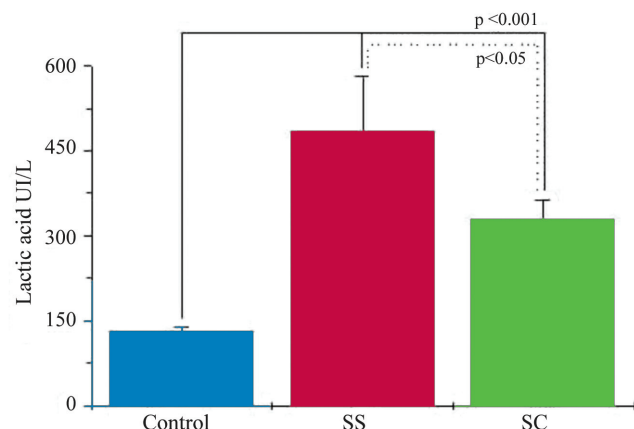


Figure 1– Blood lactate concentrations

## Discussion

The basal metabolism is essentially maintained by two energy pathways – aerobic and anaerobic. Under normal conditions, in a healthy individual the baseline metabolism is maintained by a predominance of the aerobic pathway, in which the energy substrates involved are basically the lipids and carbohydrates. It is known that there is a higher use of lipids compared to carbohydrates and that the end result of aerobic energy production consists of water (H<sub>2</sub>O), carbon dioxide gas (CO<sub>2</sub>) and adenosine-triphosphate (ATP).<sup>(23,29)</sup>

Furthermore, under normal conditions, the proportion of ATP used from the anaerobic pathway increases, as

exercising becomes more intense. In very intense exercising, that is to say, over 80% of direct maximum oxygen consumption (VO<sub>2</sub>max) or above 85% of the maximum heart rate is obtained, the anaerobic pathway begins to provide more energy than the aerobic pathway. However, different to the aerobic pathway, the lactic anaerobic pathway produces ATP and lactic acid as the end products, with the latter being transformed into lactate and released into the bloodstream.<sup>(30,31)</sup>

In a healthy body, all the lactate produced is buffered in the kidneys, heart muscle and mainly in the liver. In the kidneys, lactate binds to bicarbonate and is transformed into sodium lactate, whereas in the heart muscle and liver tissue, two molecules of lactate (C<sub>3</sub>H<sub>6</sub>O<sub>3</sub>) are transformed into glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) and this is re-used by the body.<sup>(32)</sup> When lactate production occurs at a higher rate than can be buffered, it accumulates both in the active tissues and in the bloodstream. This sets off metabolic acidosis and causes a reduction in the hepatic glucose activity and the capacity of intramuscular calcium to bind with troponin. Troponin is a contractile protein; when it binds with calcium, myosin can bind to actin, thus stimulating muscle contraction. This explains in part why the accumulation of lactate is one of the biochemical mechanisms that leads to muscle fatigue.

It is also important to point out that lactate production is directly proportional to CO<sub>2</sub> production, and consequently to a reduction in the supply of oxygen (O<sub>2</sub>) to the cells. As the demand for O<sub>2</sub> is greater than the supply, the oxidative aerobic mechanism is then supplemented by the anaerobic mechanism,<sup>(33)</sup> a metabolic deviation from aerobic to anaerobic.

From the results shown in Figure 1, it is observed that at rest, the lactate levels for both Hb SS and HB SC volunteers were higher than the control group. This finding may be explained in two ways: by the increased lactate production reflecting the deviation from aerobic to anaerobic metabolic predominance or by a reduction in the lactate buffering capacity.

Two studies reported that the mean baseline lactate levels for sickle cell disease individuals were higher than the normal values and above the lactate threshold.<sup>(34,35)</sup> It was found that the value found in these patients was 660 - 7760 IU/L, well above the mean of the present research, which was 330 IU/L for Hb SC individuals and 486 IU/L for Hb SS individuals. However, these two studies analyzed blood lactate of sickle cell disease patients with acute or chronic

hepatic impairment; some also had kidney dysfunction and thus different from the sample of this study, in which only patients who did not present these conditions were selected. These results highlight a strong correlation between the accumulation of blood lactate and the hepatic lactate metabolism.

This reinforces the idea that, irrespective of hepatic or renal involvement, sickle cell disease individuals with the SS or SC genotype have a baseline metabolic deviation of the aerobic to the anaerobic pathway, possibly caused by an adaptation to the reduced O<sub>2</sub> bioavailability to the cells of the body as a result of the sickling process. Sickling causes a reduction in the half life of red blood cells, diminishing their absolute number in sickle cell disease patients compared to healthy individuals and consequently O<sub>2</sub> transport is reduced.<sup>(27)</sup>

Moreover, it is known that elevations in the blood lactate level have been considered an indication of an increase in anaerobic metabolism of the muscle because of the low levels of O<sub>2</sub> in its cells.<sup>(27)</sup> From this finding we may also infer that the aerobic functional capacity measured by VO<sub>2</sub>max is diminished in sickle cell disease patients, not only because of cardiovascular or pulmonary involvement,<sup>(6)</sup> but also because of presenting an altered basal metabolism, that is to say, permanently in metabolic acidosis.

Metabolic acidosis is a very frequent disorder in patients hospitalized in intensive care units and is associated with elevated mortality,<sup>(36)</sup> although the prognoses of these patients depend a great deal more on the severity of the disease underlying the acid-base disorder than on the severity of the metabolic acidosis itself.<sup>(37)</sup> It is well known that extreme levels of acidemia cause various undesirable effects on cell function. Among these effects one may point out weakness and fatigue of the respiratory musculature,<sup>(38)</sup> an increase in insulin resistance and consequent increase in protein catabolism,<sup>(36)</sup> which may explain the sarcopenia observed in sickle cell disease individuals, reduction in cardiovascular response at effort and reductions in myocardial contractility,<sup>(6)</sup> as well as increases in pulmonary vascular resistance. This explains the high number of sickle cell disease patients that develop pulmonary hypertension.<sup>(38)</sup>

Finally, it can be affirmed that the more severe the sickling of cells is, the higher the basal lactate level will be; this is proven when the mean lactate value of SS subjects is compared with that of SC subjects, as both suffer from vaso-occlusive phenomena, although normally the symptoms are milder in SC subjects.<sup>(28)</sup>

## Conclusion

The resting blood lactate value in individuals with Hb SS and Hb SC diseases, but without hepatic involvement, is 4 and 3 times higher, respectively than that of healthy

subjects, possibly caused by a deviation from the predominantly aerobic to the anaerobic metabolic pathway as a result of the sickling process from which these individuals suffer.

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