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Glucose-6-phosphate dehydrogenase (G6PD). Response of the human erythrocyte and another cells to the decrease in their activity

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SUMMARY

Glucose-6-phosphate dehydrogenase is the first enzyme in the pentose phosphate pathway and the main intracellular source of reduced nicotinamide adenine nucleotide phosphate (NADPH), involved in diverse physiological processes such as antioxidant defense, (for instance in the erythrocyte) endothelial growth modulation, erythropoiesis, vascularization and phagocytosis. G6PDH deficiency is the most common X-chromosome-linked enzymopathy in human beings. Although it is present in any type cell, its absolute deficiency is incompatible with life. According to WHO, 400 million people are affected by G6PD deficiency in the world but in Colombia, the severe form prevalence is about 3% to 7%. There are no data related to slight and moderate alterations, that also have clinical effects. This paper reviews some G6PD biomolecular aspects, its classification according to activity and electrophoretic mobility, as well as some main clinical aspects related to its activity alteration.

Keywords: Erythrocyte; Physiology; Genetics; Epidemiology; Glucose-6-phosphate dehydrogenase deficiency; Congenital hemolytic anemia.

Glucosa-6-fosfato deshidrogenasa (G6PD). Respuesta de los hematíes y otras células humanas a la disminución en su actividad

RESUMEN

La glucosa-6-fosfato deshidrogenasa (G6PD) es la primera enzima de la vía pentosa fosfato y la principal fuente intracelular de nicotinamida adenina dinucleótido fosfato reducido (NADPH), compuesto comprometido en diversos procesos fisiológicos, por ejemplo defensa antioxidante (sobre todo células como los eritrocitos), modulación del crecimiento endotelial, eritropoyesis, vascularización y fagocitosis. La deficiencia de G6PD es la enzimopatía ligada al cromosoma X más común en el ser humano. Si bien se puede presentar en cualquier tipo de célula, su carencia absoluta es incompatible con la vida. Según la OMS, en el mundo hay más de 400 millones de personas afectadas por la deficiencia de la enzima, y para Colombia calculan una prevalencia de la deficiencia severa entre 3% y 7%, pero no se conocen los datos relativos a las alteraciones leves y moderadas, que también tienen efectos clínicos. El presente artículo revisa los aspectos biomoleculares más importantes de la enzima, su clasificación de acuerdo con la actividad y la movilidad electroforética, y también se mencionan algunos aspectos clínicos relacionados con la alteración de su actividad.

Palabras clave: Ultraestructura; Fisiología; Genética; Epidemiología; Deficiencia de glucosa-6-fosfato deshidrogenasa; Eritrocitos; Anemia hemolítica congénita.

All living organisms, be yeasts or protozoa, plants or animals express the glucose-6-phosphate dehydrogenase enzyme (G6PD)¹.

Though G6PD is found in the cytoplasm of all mammals cells, its deficiency is more evident in the red blood cells

probably because these cells live without nucleus for a long time and because they contain proteases that degrade the mutant enzyme in major degree than other proteases in other tissues¹.

Since the erythrocyte is a cell that transports oxygen by

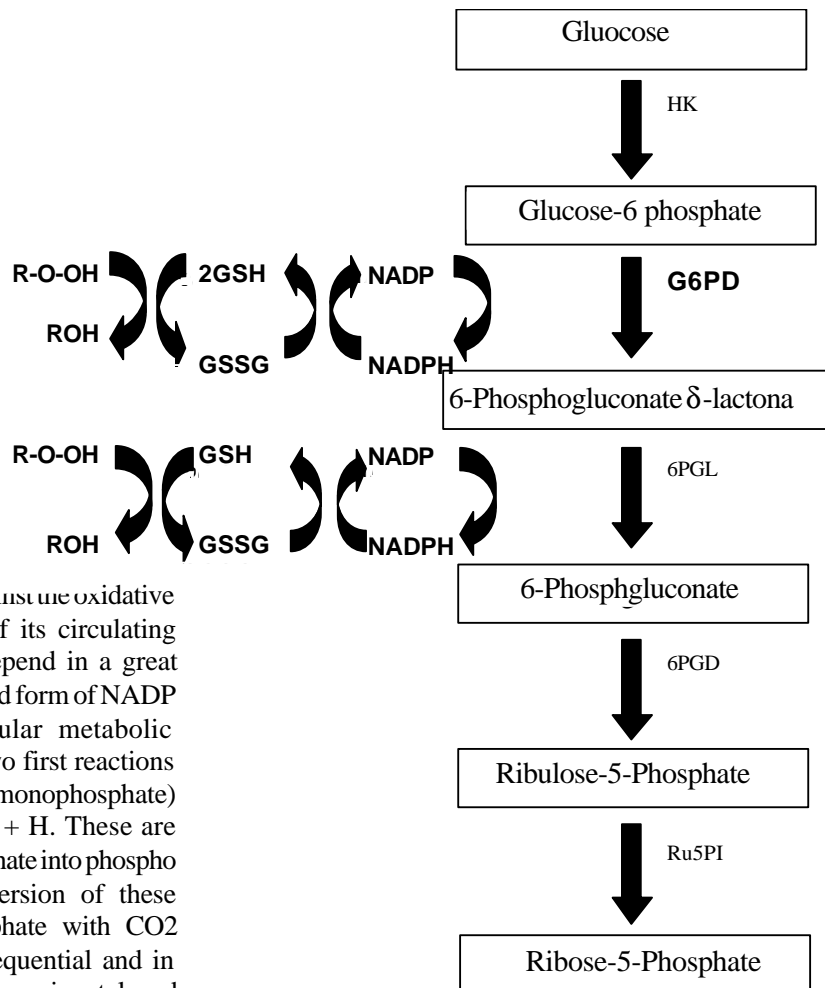
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Figure 1. Action of G6PD in the hexose monophosphate. NADPH participates in the reduction of the toxic peroxide (R-O-OH) through the glutathione (GSH and GSSG).

HK=Hexokinase
 6PGL=Phosphogluconactonase
 6PGD=6-Phosphogluconate dehydrogenase
 Ru5PI=Ribulose 5-Phosphate isomerase
 GSH=Reduced glutathione
 GSSG=Oxidated glutathione
 R-O-OH=Peroxides



excellence, its mechanisms of defense against the oxidative stress make part of the maintenance of its circulating activity. These defense mechanisms depend in a great part on the metabolic supply of the reduced form of NADP ($\text{NADPH} + \text{H}^+$). Due to the particular metabolic characteristics of these cells, only the two first reactions of the pentose via (also called hexose monophosphate) have the capacity to generate $\text{NADPH} + \text{H}^+$. These are first the conversion of the glucose-6-phosphate into phosphogluconic acid-6 and second the conversion of these intermediate one into ribulose-5-phosphate with CO_2 detachment. These two reactions are sequential and in both the NADP is reduced. While the first one is catalyzed by the G6PD enzyme, the second one it is by the 6-phosphogluconate dehydrogenase.

Through the production of NADPH the erythrocytes reduce the oxidative glutathione to reduced glutathione which process is catalyzed by the glutathione reductase enzyme which is a flavoprotein with FAD (flavin adenin dinucleotic). At the same time the reduced glutathione retires the H_2O_2 , from the erythrocyte in a reaction catalyzed by the peroxidase glutathione. This reaction is important because the H_2O_2 might reduce the life expectancy of the erythrocytes for the increase in the speed of hemoglobin oxidation to metahemoglobin² (Figure 1).

G6PD deficiency produces irreversible oxidative damage and cell death³. The average life of 60 days of the enzyme reflects stepwise the age of the red blood cells. This way to major age the activity of some enzymes

decreases since the erythrocytes are unable to synthesize new protein molecules. For this reason, the reticulocytes have an enzymatic activity five times major than that of the senescent⁴ red blood cells and they must be separated before determining the enzyme activity.

Structure. The Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49; D-glucose-6-phosphate: NADP oxidoreductase)⁵ enzyme is present in all cells. In the erythrocytes it is found in its dimeric and tetrameric forms. The monomer has a molecular weight of 59,256 daltons and counts with 515 amino acids. The catalytic activity is only initiated when an association is established, in balance status, between the dimeric and tetrameric⁶ forms.

Such an association requires the NADP presence, strongly tied by the enzyme^{7,8} which makes that NADP performs a dual role not only as structural component but

as a coenzyme⁹⁻¹¹. In 1967, Luzzatto¹² postulated at least two sites of union of the NADP to the enzyme, with a condition of transition from low to high affinity when NADP's concentration increases, which means that there exist two possible forms of the enzyme, according to its affinity by the NADP. These conditions might change in case of appearance of a competitive inhibitor of the NADPH. The low constant of dissociation for the NADPH suggests that the reaction can be a very efficient controller, acting as a feedback, which would regulate the enzymatic activity. These facts allowed to establish that the relation of NADP/NADPH concentration is a regulatory mechanism of the activity of the G6PD and therefore of the hexose monophosphate (HMP) in the red blood cells.

Function. The importance of the G6PD is found in the transcendence of the cellular processes in which it takes part, such as:

NADPH genesis effected from the first two steps of the hexose monophosphate. The NADPH takes part in the biosynthesis that reduces cholesterol and oil acids and also in the synthesis of the nitric oxide (NO). On the other hand it is needed for the activity of the metahemoglobin reductase and for the maintenance of the reduced level of glutathione (GSH). NADPH and GSH are responsible for the potential redox effective to protect from the oxidative stress the groups of sulfhydryl of the cellular membrane, as well as the enzymes and the hemoglobin that compromises erythrocyte survival⁸.

Other functions that show the transcendence of this enzyme in the cell life are the following:

1. Regulation of the activity of the KU protein implied in repairing the DNA after the damage that radiations cause. The intervention of the G6PD is effected through the pentose cycle and consists of facilitating the union of KU - with reduced cysteine residues- to the DNA in repairing process¹³.
2. Early development of the embryo. When there is a severe G6PD deficiency in the extra-embryonic tissues, the placenta development stops and the embryo dies¹⁴.
3. Survival of the fetus during the transition of the fetal hemoglobin to the adult form. Here the G6PD prevents the oxidative damage due to the generation of species reactive to oxygen from the adult hemoglobin^{14,15}.
4. Phagocytosis in white cells. The severe deficiency of this enzyme results in a reduction of the NADPH generation, which results in a decrease in the production of hydrogen peroxide (H₂O₂) and therefore the

neutrophil microbicide activity is affected and likewise its inflammatory response¹⁶. Though the clinical characteristics of the severe deficiency are similar to those of the granulomatosa chronic disease (EGC), its appearance happens, unlike the latter, during more advanced stages of life^{17,18}. The EGC constitutes a fundamental model to investigate the composition and the activation of the microbicide system of the phagocyte cells, especially of the neutrophil ones. This entity is caused by a deep defect in the respiratory explosion that accompanies the phagocytosis of all myeloid cells (neutrophil, eosinophile, monocyte, macrophage). The respiratory explosion generates the catalytic conversion of the molecular oxygen in the super oxide anion that leads to the hydrogen peroxide formation, the hypochlorous acid and the hydroxile radicals. These derivates from the oxygen play an important role in the microbicide reaction against bacteria and fungi^{19,20}.

5. Modulation of the vascular endothelial factor growth that regulates the angiogenesis. NADPH is used as a cofactor of the endothelial nitric oxide sintetase (eNOS). Therefore the nitric oxide required for the modulation of the growth and for the endothelial migration during the vascular growth is maintained in an adequate level¹⁷.
6. Most of the genes able to reduce the risk against certain infections as the malaria are expressed in the red blood cell, which is considered like a genetic and/or evolutionary mechanism of defense, as in the case of the genes that express the G6PD²¹.

Deficiency. The G6PD deficiency still prevails as the most common of all the inherited enzymatic defects^{22,23} and it is clinically significant not only in the hematological field but also in the human biology²⁴ and it is characterized by a large biochemical and genetic heterogeneity. The deficiency of G6PD has been the prototype within the hemolytic anemias due to an enzymopathy as a primary abnormality of the erythrocyte. Likewise it is an example of hemolytic anemia due to an interaction between extra cellular and intracellular causes, since hemolysis in most cases is triggered by exogen agents²⁵.

Hemolysis of deficient red blood cells occurs as a consequence of the increase in the susceptibility to the oxidative damage due to the incapacity of cells to reduce the NADP to NADPH in a normal way. In presence of oxidant agents the NADPH production through the HMP is stimulated multiple times so that the NADPH and GSH

levels maintain stable. These events are due to the over-expression of G6PD²⁶. The exact mechanism involved in the increase of the sensibility to the oxidative damage, facilitator of hemolysis, is not clear yet. However, there exists a significant volume of information on the favism, which is higher than the available one about the different medicines that might produce it. Faba beans contain compounds such as divicine and isouramil that produce irreversible GSH oxidation as well as in other groups of proteins united by SH groups. This favors not only an electrolytic unbalance in the red blood cells, but also the union by the intercrossing of membranes and microvesiculation, events accompanied by an increase in the calcium concentration in erythrocyte²⁷.

The G6PD deficiency is produced by different genetic mechanisms such as deletions, precise mutations and substitutions that affect the transcription, process or primary enzyme structure, which functionally results in a decrease of the enzymatic activity or loss of affinity by the substrate. There are other factors that influence the activity of the enzyme. Thus, in a study whose objective was to determine the possible relation between the activity of the G6PD enzyme and the hypoxia, it was found that the hypoxia favored a decrease in its activity^{27,28}.

Variants. The true deficiency of G6PD was identified initially in the middle of the last century, in black race North americans during the investigations carried out over the hemolytic effect of the primaquine¹⁹. At the present time such medicine continues being a causal agent of the deficiency in Iraqi soldiers with malaria²⁹.

From mid of the last century, it was accepted that the primary metabolic defect in individuals susceptible to the hemolysis secondary to medicines or to the faba bean consumption (*Vicia faba*), corresponds to a low activity of the G6PD in erythrocytes³⁰. Although the association between the deficiency of G6PD and the non-immune hemolytic and the non-spherocytic anemia²² is clearly defined, also it is evident its correlation with the hemolysis due to medicines, food and to other events such as infection processes, situation outlined by Vulliamy *et al.*³¹ as the most important cause of hemolysis. Towards 1958, Gross *et al.*³⁰, on one hand and Szeinberg *et al.*²² on the other one, determined that the enzymatic deficiency had a hereditary base and suggested that it was bound to sex. The biochemical characterization allowed identifying not less than 442 variants of the deficiency of the enzyme. About 229 of them were described by methods used by the

expert group of the World Health Organization (WHO). On the other hand 60 mutations or their combinations were documented, all of precise nature taking into account that the total deficiency is incompatible with life²³.

According to its activity level the enzyme variants were classified in five types²³, such as:

Class 1: Deficiency of the enzyme with chronic non-spherocytic-hemolytic anemia (CNSHA).

Class 2: Severe Enzymatic deficiency (less than 10%, for example the mediterranean form).

Class 3: Moderate enzymatic deficiency (10%-60%, for example, the African form).

Class 4: Low or absent enzymatic deficiency (60%-100%).

Class 5: Enzymatic activity above normal rates.

The class 1 variant is a rare and severe one, associated with chronic non-spherocytic hemolytic anemia. Of sporadic appearance, their cases are considered unique³¹. In regions such as the African and Asian continents and the Mediterranean river basin there exists a high frequency of the different variants of the enzymatic deficiency, whereas in China and Japan the frequency is low²³.

In the Mediterranean populations the enzymatic deficiency is much more severe and frequent than in the population of North American black race³², where the defect was identified in the red blood cells.

In contrast, this one was found in several diverse cellular types, obtained in Italian sensible individuals and of Jewish race³³. With respect to the frequency of the severe deficiency, the variation between the different populations is notorious. Thus, among the black race Americans, the frequency of the gene of the enzymatic deficiency is from 0.10% to 0.11%³⁴ with 15% of enzymatic activity related to normal³⁵. As an example of an elevated frequency of the deficiency, we may mention the Kurd Jews where it reaches, in its Mediterranean form, an equal value to 0.70%³⁶. The Mediterranean form is a variant whose frequency of polymorphism has an activity lower than 10%. In it, the mutation appears in the 188 amino acid, with substitution of phenylalanine (Phe) by serine (Ser)³⁷.

In Saudi Arabia the most frequent variant is the Mediterranean one, with frequencies that oscillate between 0% and 0.4% in men and 0% and 0.2% in women. It is possible that the high prevalence in women obeys to a uniparental dysomia or, to the high existing consanguinity or to that the chromosome X containing the normal gene is the one that inactivates itself during the genetic imprints³⁸.

In Latin America some variants of the enzyme have been described. In Mexico for example, 18 were identified, which are also of common appearance in other regions like the African continent, the south of Europe and the Southeast Asia³⁹. Whereas in Mexico the frequency of the deficiency was between 0.4% and 4.1%, in Cuba it was 4.9% with a prevalence of the variant A-, and 7% for the A+ variant⁴⁰. For countries like Colombia, the frequency calculated by the WHO for the variants phenotypically associated with the severe deficiency (Type 2, with lower activity than 10%) is between 3% and 7%²³. Nevertheless, in a survey developed among 103 individuals of masculine sex, donors of the Blood Donation Point of the Colombian Red Cross and in appearance healthy, it was found a frequency of subnormal activity (<60%) of approximately 19.4%. This research was developed between June and October of 2003, by means of the application of the qualitative technique of Beutler E (Palomino F. 2003. Universidad Nacional de Colombia, Personal communication).

Another classification is realized by comparing the electrophoretic mobility of the different variants with the normal B enzyme, being the variant A- enzyme, present in individuals of black race with low enzymatic activity, faster in alkaline pH than the normal enzyme, in contrast, the variant of the Mediterranean deficient individual's moves at a normal speed³⁷. Another frequent variant, A+, has a normal activity and it is found in about 20% of the North Americans of black race. This variant is electrophoretically faster than the B, fact that is understood taking into account that the substitution of asp (neutral amino acid) by asn (acid amino acid) in 126 positions modifies the enzyme electrical charge, which is reflected in a faster electrophoretic mobility³⁷.

The variant A- is found in near 11% of the North American black population. However, its frequency is greater in the African sub-saharan black population. The enzymatic activity of this variant corresponds to 5% and 15% of the normal one, this decrease is due to the presence of two substitutions, not of one as it happens in the A+ variant. One of these substitutions is similar to the one that appears in variant A+ and the other one, unique for this variant, obeys to the change of the val for met in the 68 position⁴¹.

Genetics. The G6PD enzyme is codified by a gene that is present in the terminal region of the long arm of chromosome X, (Xq28), less than 2 centi-Morgan to the gene of factor VIII. In men, the hereditary condition linked

Table 1
Molecular characteristics of the G6PD gene

DNA	Localization	Xq2.8
	Gen size (in kilobasis)	18.5
	Number of exons	13
	Number of introns	12
mRNA	Size (in nucleotides)	2269
Protein	Number of amino acids	515
	Molecular weight (in daltons)	59,265
	Subunits by molecule of	
	Active enzyme	2 or 4

to X determines its hemizygotic character, which means that there is a single one allele, due to the absence of locus homologous. There are also homozygotic women in populations where the frequency of the G6PD deficiency is high. Hemocytotic women are carriers although they might develop hemolytic attacks. The G6PD gen has been mapped in the distal part of the long arm, its length is 18 Kb and counts with 13 exons⁴² (Table 1).

The region of the gene to be codified for the protein includes 12 segments, with an average size between 12 and 236 bp and one intron present in the non-translator region 5'. In many cellular lines, the greater end 5' of the G6PD mRNA is located at a distance of 177 bp «upstream» from the transcription initiation codon^{43,44}. Although the mutations extend throughout the coding region of the gene, there exist few (4 of 56) that give origin to the most severe form of deficiency of the enzyme, this is, the one that is associated with CNSHA (type 1) in the 160 amino acids of the N-terminal end. However, there is no one that causes moderate forms of deficiency (types 2 and 3) in the 48 amino acids of the C-terminal end. Many variants in this region exhibit abnormal electrophoretic mobilities and are particularly unstable when the NADP concentration is low. This is because this region codifies for the domain of the union to NADP⁴⁵.

In the A- variant a substitution identical to A+ is present, although there is a second substitution in the 202 G --->A nucleotide of exon 4 which results in the change of val for met, accompanied by the instability of the enzyme *in vivo*. Therefore the difference between forms A and B corresponds to the amino acid that occupies the 126 position, probably as a result of an alternate join or «splicing» of considerable heterogeneity among the different

cDNAs of the G6PD⁴¹.

Clinical manifestations. Almost all the people who attend with the G6PD deficiency are usually asymptomatic and the disease is only pronounced when they ingest drugs or chemicals that trigger the intravascular massive hemolysis. The clinical expression then results from the interaction of the molecular properties of each G6PD variant and from exogenous factors. Different clinical syndromes associated with the deficiency of this enzyme have been described and they include:

Hemolysis induced by drugs. Classically, after ingestion of certain agents like sulfamides, antipyretics, nitrofurans and antimalarial medicines, like the primaquine and chloroquine, the patient develops fever, black color urine, ictericia and anemia. The acute tubular necrosis might complicate the severe hemolytic episode, mainly in the underlying diseases of the liver, like hepatitis. The maintenance of the adequate renal flow of the blood, by forced alkaline diuresis, might prevent this complication. In these cases with the committed renal flow of the blood as evidenced by the low flow of urine, the transfusion is the ideal in order to eliminate the damaged red cells that block the microcirculation and can also avoid the renal complication. In some patients, the disseminated intravascular coagulation (DIC) might complicate the massive intravascular hemolysis and might need the appropriate treatment⁴⁵.

Hemolysis induced by infection. The infection is perhaps the most common cause in patients with G6PD deficiency. The mechanism of hemolysis induced by infections is not well known yet; an explanation may be that the generation of hydrogen peroxide by the polymorphonuclear neutrophils might cause a decrease in the quantity of reduced glutathione, whose function is to eliminate, from the red cell, the accumulated metabolites that oxidate the sulfhydryl groups formed by the oxidative stress and therefore decreasing the protective capacity of the cell. On the other hand, the activation of the neutrophils takes part directly in the peroxidation of lipids of the membrane and causes directly the destruction of the cell⁴⁶. The severity and clinical consequences are influenced by many factors that include the simultaneous administration of oxidating medicine, the previous levels of hemoglobin, the hepatic function and the age⁴⁷.

Favism. It is recognized from the antiquity; patients present a clinical picture similar to the induced by drugs, that is triggered within the following 24 to 48 hours after

the ingestion of faba beans. It is characterized by the presence of a picture of acute hemolysis after ingesting faba beans; however, not all individuals with G6PD deficiency present hemolysis when ingesting faba beans⁴⁸.

Favisms symptoms are developed a few hours after the ingestion. The most common are nausea, vomit, discomfort and dizziness. After these symptoms there occurs an acute hemolysis where frequently the recount of erythrocytes falls below $1.0 \times 10^{12}/l$. In most red blood cells Heinz bodies appear. The hemoglobinemia and hemoglobinuria are present. Symptoms stopped generally after 2 to 6 days^{49,50}.

Nonspherocytic chronic hemolytic anemia. The variants of type I, are characterized by this finding, due to the so severe degree of enzymatic deficiency. The hemolysis is only partially intravascular and it is possible to be accompanied by biliary calculous and splenomegaly. Nevertheless, it exists a variability in the manifestations associated with this type of chronic anemia^{47,50,51}. At the same time there are reports on cases in literature where they describe to chronic hemolytic anemia and a cause, although rare, is the G6PD deficiency⁵².

Preeclampsia. Entity apparently associated in a partial form with a lipidic peroxidation of the plasmatic membrane of the syncytiotrophoblast. For that reason, patients with some alteration in the G6PD activity might have serious difficulties in the reduction of the GSSG to GSH and thus present alterations in the antioxidant defense⁵³.

On the other hand, it is interesting to outline how the G6PD deficiency (variant A-) is associated with an increase in the resistance to the infection caused by *Plasmodium falciparum* in the sub-Sahara region of Africa. This indicates that there is a strong adaptation response in front of this red blood cells invasion⁵⁴.

CONCLUSIONS

Considering that Colombia has been seat of multiple migrations from Africa and Europe, it is necessary to develop surveys in order to evaluate the rank of activity of the G6PD, as well as its possible variants in this region. The obtained knowledge will be able to complement the findings collected in other latitudes and therefore it will derive in a better diagnosis, approach and treatment of the diseases associated with the hemolysis and the oxidation, like anemia, diabetes, arterial hypertension and cancer among others. On the other hand, this knowledge might be

applied in the area of the physiology of the exercise, where, because of the oxidative stress, it could happen from hemolysis to the sudden death of the sportsman.

Considering the increasing findings on the participation in certain pathologies, as well as in the understanding of evolutionary processes of the human species, the determination of the activity of this enzyme is a priority for populations like the Colombian one where there are only some extrapolated data obtained by WHO.

In different parts from the world different techniques arise that intend to favor the fast determination with high sensitivity and specificity of the activity of this enzyme^{55,56}. However, In Colombia only some determinations have taken place in small groups (data without publishing), and there exist small work groups from certain universities like the Universidad del Rosario of Bogotá's, who will soon give their results in relation to the prevalence of this alteration in the metabolism of hereditary type.

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