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Full Length Research Paper

Altered plasma hexose sugar metabolism in sickle cell anaemia

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Theoretical suggestion that the metabolism and plasma levels of some hexoses (glucose and fructose) in sickle cell anaemia could be altered was investigated. The levels were determined for 35 normal (HbAA) and for the three sickle cell states (32 HbAS; 35 HbSS, and 33 HbSS-in-crisis, HbSSc). The enzymatic glucose-oxidase method was employed in glucose estimation. The reaction of fructose with indole-3-acetic acid to yield a coloured product was exploited in the estimation of fructose. Mean plasma glucose was found to be highest in the HbSSc state (84.80 ± 4.10 mg/dl), followed by HbSS (78.59 ± 4.20 mg/dl), HbAS (74.80 ± 6.20 mg/dl) and lowest in HbAA (70.10 ± 0.05 mg/dl). The differences between the normal and all the disease states are significant (between HbAS and HbAA, for example: t = 2.2717; df = 65; p < 0.05). The reverse order was observed with respect to fructose concentrations: lowest in HbSSc (0.99 ± 0.05 mg/dl), HbSS (1.09 ± 0.05 mg/dl), HbAS (1.25 ± 0.05 mg/dl), and highest in HbAA (1.32 ± 0.08 mg/dl). Regression of glucose on fructose showed positive correlation in the HbAA group (r = 0.7900; df = 33; p < 0.001), no correlation in the HbAS group (r = - 0.0193; df = 30; p > 0.20), and negative correlation in the HbSS group (r = - 0.3191; df = 65; p < 0.001). The differences between the 'Glucose-to-Fructose Ratio' for the different states are very significant (p<0.001) and could serve as biomarker of sickle cell anaemia intensity.

Key words: Hexose, anergy, glucose, fructose, spermatozoa, energy metabolism, metabolic control, sickle cell anaemia, glucose-to-fructose ratio.

INTRODUCTION

Evidence exists to indicate increase in resting energy expenditure in sickle cell disease (Singhal et al., 2002). This agrees with the finding that sickle cell anaemia is very energy-costly, as a result of high entropy in some body system functions (Osuagwu, 2007). There is also evidence that alteration in pattern of nutrient metabolism results in this increase in resting energy. There is increase in protein energy metabolism, as compared to lipid and carbohydrate (Borel et al., 1998). A number of observed factors are suggestive of the possibility of the alteration of the patterns of utilization of plasma hexoses (glucose and fructose), in sickle cell disease (Lachant et al., 1983). These observations include the reduced activities of many of the enzymes of the glycolysis pathway, including the key ones such as hexokinase, as noted by Zerez and Tanaka (1994). The deficiency of glucose-6-phosphate dehydrogenase, a key enzyme of glycolysis is already known (Yoshida, 1973). And so is the build-up of lactic acid, an end-product of glycolysis, which could impact on the glycolysis system by negative feedback (Pattillo and Gladden, 2005; Sara et al., 2006). Zinc deficiency is known to occur in sickle cell disease (Ballester and Prassad, 1983). Zinc is a known co-factor in some of the metabolic processes of glucose metabolism, including key role in insulin activity (Chausmer, 1998). Zinc defi-ciency would negatively impact on the glucose metabolic process. Some of the symptoms of diabetes like leg ulceration, lactic acidosis, and anergy are observed in sickle-cell disease (Ballester and Prassad, 1983). This suggests the possibility of deranged glucose metabolism, as in diabetes. There is evidence of the involvement of the insulin-producing pancreas in the pathophysiology of sickle cell

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Table 1. Plasma glucose and fructose levels and ratios in sickle cell states.

Sickle Cell State	Number of Subjects in Group	Plasma Glucose Level, mg/dl	Plasma Fructose Level, mg/dl	Glucose/Fructose Ratio
HbAA	35	70.10 ± 7.50	1.32 ± 0.08	53.11
HbAS	32	74.75 ± 6.20	1.25 ± 0.05	59.80
HbSS	34	78.59 ± 4.20	1.09 ± 0.05	72.10
HbSSc	33	84.80 ± 4.10	0.99 ± 0.04	85.66

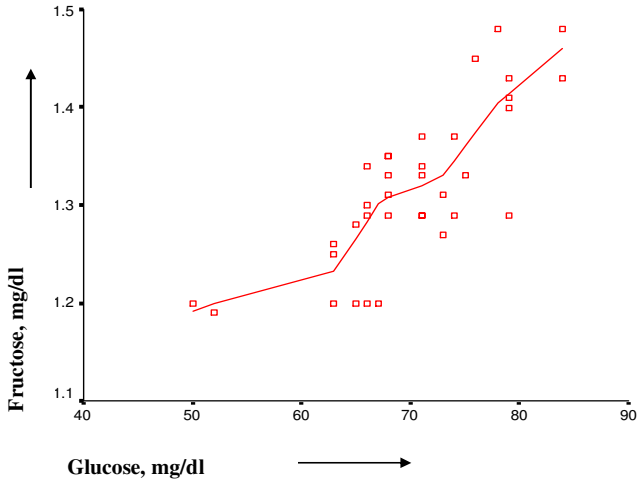

Figure 1. Plasma glucose to fructose in HbAA.

anaemia, with observed lesions, such as islet cells dispersion, nesidioblastosis and increased size in young sufferers (Culberston et al., 2001). Adekile and co-workers (1985) had demonstrated marginally higher glucose intolerance in children with sickle cell anaemia; their work had suggested “a primary impairment of insulin secretion”. They had also noted earlier reports that adult sicklers appear more glucose intolerant than normal. Fructose and glucose mutually influence each other’s metabolism (Zierath et al., 1995). This study investigates the possibility of significant alteration in the metabolism of these two isomeric hexoses that are important in glycolysis, as well as general energy metabolism. Their plasma concentrations are assayed in three defined sicklecell states and compared to that from the plasma of normal individuals.

MATERIALS AND METHODS

Blood samples used in the study were collected from informed and consenting volunteers as well as consenting patients who attended the sickle cell clinic at Community Hospital Osina, Imo State, Nigeria. The consent of parents was sought on behalf of their children. Fasting blood was drawn from each subject, the plasma extracted, and the assay carried out immediately to avoid metabolic alteration of hexose levels due to long storage. The number of males and females in each study group was made as equal as possible to minimize systematic bias due to sex differences in metabolism. A total of 2ml of blood was collected from each subject. The blood sample was split into two; 0.5ml was put into a tube containing 1.5ml normal saline, hemolysed with water and used for the electrophoresis test to confirm the hemoglobin genotype of each subject. 1.5ml of the blood sample was put in a bottle containing one drop of fluoride-oxalate; this was centrifuged at 3200 rpm for 2 min to extract the plasma for the assays.

Glucose levels in the plasma sample were estimated by the enzymatic glucose-oxidase method, employing *GLUCOSA* croma-test-kits from Linear Chemicals, Badalona, (Spain). Plasma fructose concentration was estimated by the 3-indoleacetatic acid method of Karvomen and Malan (1955), as modified by Ojiako and Akubugwo (1997), to generate a purple colour whose intensity, at 520 nm, is proportional to fructose concentration.

RESULTS AND DISCUSSION

Table 1 is a summary of the result of the assays. It shows the tendency of glucose concentration to rise with intensity of the sickle cell condition, while the fructose concentration tends to decrease. Considering the fact that the erythrocyte depends on glucose metabolism for its energy, this is a potentially serious problem.

It is observed that the mean glucose levels obtained in this investigation for HbAA (70.10 mg/dl) and HbSS (78.59 mg/dl) is comparable to that obtained in 1985 (72 and 81 mg/dl) by other Nigerian workers (Adekile et al., 1985). Although they worked with a smaller number (12 HbSS and 9 HbAA), which would make statistical assessment more difficult. Significance test of the observed differences between the normal and sickle cell states showed that between HbAS and HbAA was significant ($t = 2.2717$; $df = 65$; $p < 0.05$), as others. HbSS to HbAA ($t = 5.8036$; $df = 67$; $p < 0.001$), HbAA and HbSSc ($t = 9.9398$, $df = 66$, $p < 0.001$). The level of plasma fructose difference between normal and the sickle cell states was also significant (for HbAA and HbAS, $t = 4.2073$, $df = 65$, $p < 0.001$; HbAA and HbSS, $t = 13.3795$, $df = 67$, $p < 0.001$; HbAA and HbSSc, $t=20.3540$, $df = 66$, $p < 0.001$). These relationships are presented in Figures 1, 2 and 3 respectively. Regression of fructose on glucose for each of the groups show a positive correlation in the HbAA state ($r = 0.7900$, $df = 33$, $p < 0.001$), no correlation in the HbAS state ($r = - 0.0193$, $df = 30$, $p > 0.20$), a negative correlation in the sickle cell group (HbSS and HbSSc, together) ($r = -0.3191$, $df = 65$, $p < 0.01$), respectively. It

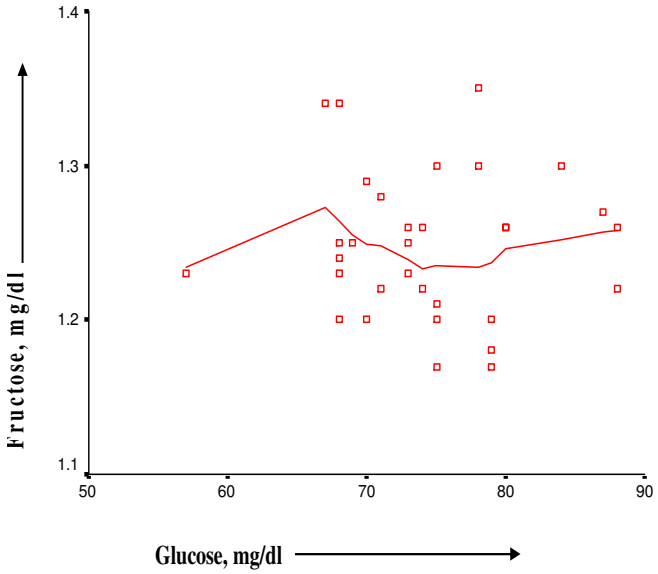

Figure 2. Plasma glucose to fructose levels in HbAS.

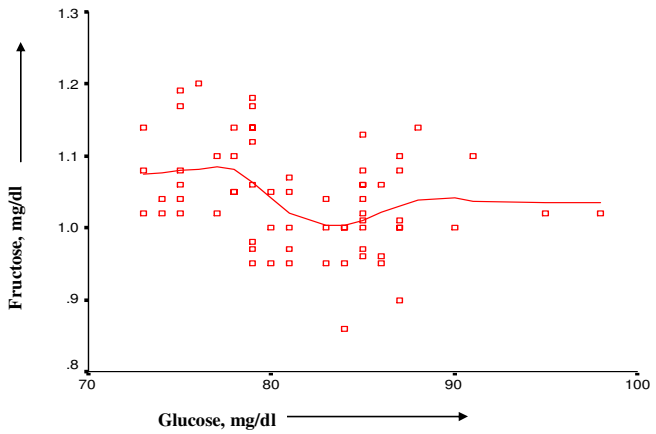

Figure 3. Plasma glucose to fructose levels in HbSS.

It can be deduced from this result that the utilization of these two hexose sugars are altered in sickle cell disease; the utilization of glucose is reduced as fruc-tose is increased. This suggests a case of derangement of hexose metabolism in sickle cell anaemia. Carriers of the sickle cell gene appear more susceptible to impaired glucose metabolism than non-carriers. Plasma fructose depletion in sicklers could be compensation for dimi-nished glucose utilization because of the known greater ease of fructose migration into cells, as it is not under metabolic control (http://www.medbio.info/Horn/Time2012/carbohydrate_metabolism.htm). This might explain the reported useful employment of fructose in the manage-ment of sickle cell disease (Markov et al., 2001; Green, 2005). This result also suggests that cells that depend mainly on fructose for their energy need, sperm cells for example (Dandekar and Harikumar, 1997), would be jeopardized in sickle cell anaemia. The anergy observed in sicklecell anaemia could be due to this reduced capa-city to metabolize glucose. Glucose is known to increase lactic acid build-up in sicklecell disease (Markov et al, 2001). This could result to lactic acidosis, and derange-ment of some of the enzyme systems at abnormal pH.

Patterns of hexose metabolism could be employed as, possible, *biomarker* of sickle cell disease intensity. Measurements of plasma glucose and fructose levels, and their comparison to the concentrations in normal subjects could be used to estimate the intensity of a sickle cell disease condition (a ratio of plasma glucose to fructose concentrations would show sharper differences between groups, due to the inverse trends of the observed changes). A more comprehensive investigation of the altered pattern of hexose sugar metabolism in sicklecell disease is likely to shed more light on its etiology, and suggest some possible management strategies.

The result of this work suggests that low dose administration of insulin, particularly to those in crisis, could be of relief to sicklers by enhancing glucose utilization for energy generation. This possibility should be investigated.

Conclusion

Plasma hexose sugar (glucose and fructose) levels and Metabolism are altered in sicklecell disease. The essential dependence of the *erythrocyte* on glycolysis for its metabolic energy, as well as the central role of glucose as the central energy nutrient of the body makes this alteration a fundamental problem that could account for some of the observed lesions of sicklecell disease. The carriers of the sicklecell gene could be more susceptible to impaired glucose metabolism, and other disease conditions tied to the observed alteration in the patterns of metabolism of hexose sugars. The experimentally established relief that fructose supplement affords the sicklers suggests that the observed depletion of plasma fructose in sickle cell disease is a basic phenomenon that should be more systematically investigated. Degree of blood hexose (glucose and fructose) concentration altera-tion can be investigated as possible biomarker for sickle-cell anaemia intensity. The glucose to fructose ratio is a potential parameter for such assessment; the ratio amplifies the differences between sickle cell states, as it simultaneously incorporates the changes in glucose and fructose, as shown in Table 1. Dietary or infusion fructose supplementation as well as glucose regulation for sicklers is suggested to be further investigated as apart of the management strategy in sickle cell anaemia.

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