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Effect of Hyperglycemia on Erythrocyte Carbonic Anhydrase and Lactic Acid in Type II Diabetic Subjects

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Abstract

Background: Carbonic anhydrase (CA) is a ubiquitous enzyme catalyzing the reversible hydration of CO_2 to HCO_3^- and H^+. CA plays a crucial role in CO_2 transport, acid-base balance, and in linking local acidosis to O_2 unloading from hemoglobin and also facilitates lactate shuttling across the monocarboxylate transporters (MCT). The study aimed to investigate the influence of hyperglycemia on erythrocyte carbonic anhydrase activity and lactic acid in type II diabetic patients. Method: Red blood cell carbonic anhydrase activity was determined in washed lysed-hemolysate by the action of the enzyme on the substrate p-nitrophenyl acetate. The absorbance of released p-nitrophenol was recorded at 345 nm. Glycated Hemoglobin was determined by ion exchange method (Spectrum Diagnostic Kit). Blood glucose, lactate, cholesterol and triglyceride were determined using Accutrend GCT meter (Roche, Germany) with cobias® test strips. Results: The present study showed that hyperglycemia significantly ($p < 0.05$) increased both erythrocyte carbonic anhydrase activity and blood lactate level in type II diabetic patients. Conclusion: We may conclude that hyperglycemia may be responsible for the increased activity of carbonic anhydrase activity and blood lactate concentration.

Keywords

Carbonic Anhydrase, Lactic Acid, Glycated Hemoglobin, Type II Diabetes

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1. Introduction

Human erythrocytes are continuously being exposed to glucose in plasma during their circulatory life span of 120 days. The erythrocytes and their membranes have always been objects of studies as they play an important role in various physiological and metabolic events. Erythrocytes depend on the anaerobic conversion of glucose by the Embden-Meyerhof pathway for the generation of ATP. Red blood cells produce lactic acid as a byproduct of the regeneration of ATP during anaerobic glycolysis but cannot use lactic acid [1]. The rate of production can increase 50-fold if either glucose or glycogen is required to generate ATP in the absence of oxygen [1]. H^+ transport system under conditions of exclusively aerobic metabolism is used by the cell to maintain a facilitation of CO_2 diffusion, whereas under conditions of dominating anaerobic glycolysis and low intracellular pH, it is mainly used to transport H^+ along with the lactate anion through the monocarboxylate transporters (MCT), a prerequisite for the elimination of lactic acid from the cell. Increasing lactate levels were associated with increased mortality [2]. Previous studies have shown that MCT-dependent lactate-H^+ flux is facilitated by bicarbonate transporters and carbonic anhydrase activity in various cells and tissue [3] [4].

Carbonic anhydrase—a pH regulatory enzyme catalyzes the reversible hydration of carbon dioxide and the dehydration of bicarbonate ions [5] and has been demonstrated in red blood cells, kidney, the gastrointestinal tract, and other tissues of the body. Some early evidences suggest that the changes in erythrocytes carbonic anhydrase activity may be an initial step of altered metabolism in diabetes mellitus [6] [7]. Carbonic anhydrase (CA) activities have been associated with metabolic diseases such as diabetes mellitus and hypertension [8]-[11].

Metabolic acidosis is the most common serious acid-base disorder complicating diabetes mellitus. Metabolic acidosis is associated with increased mortality [12]. Lactic acidosis results in higher mortality than metabolic acidosis of a different etiology [13]. Lactic acidosis is an alarming metabolic signal of many pathological states, and endogenous clearance of lactate is a commonly used prognostic marker of illness [14]. Shapiro *et al.* [15], in a study with 1278 patients with infection, demonstrated that increasing lactate levels were associated with increased mortality. Given carbonic anhydrase association with lactate flux through the MCTs in previous studies, the study aimed to investigate the effect of hyperglycemia on erythrocyte carbonic anhydrase changes in diabetes.

2. Materials and Methods

2.1. Ethical Approval

The study was approved by the Research Ethical Committee, of Kano State Hospital Management Board, Nigeria.

2.2. Subjects

Ten Female black African patients with type 2 diabetes and ten female black African healthy control subjects without diabetes participated in this study. The average age group of diabetic patients ranges from 25 to 50 years, average weight of 50 to 70 kg. Dietary supplementation and lifestyle differences were not considered in this study. Blood from fasted type II diabetic patients (n = 10) attending Diabetic Clinic at Nassarawa Specialist Hospital Kano, Nigeria was obtained. Written informed consent was obtained from all subjects after the procedure had been fully explained. Blood from fasted healthy subjects (n = 10) was also obtained from the Dept. of Chemistry Ahmadu Bello University, Zaria, Nigeria. Written informed consent was obtained from all subjects after the procedure had been fully explained.

2.3. Hemolysate Preparation

The blood samples were centrifuged at 3500 rpm for 10 minutes. The plasma was separated from the cells and buffy coat removed. The packed red cells were washed three times with normal saline (0.9% NaCl) and were lysed with ice cold water, yielding destroyed plasma membranes.

2.4. Measurement of Erythrocyte Carbonic Anhydrase

Assay of Crude erythrocyte Carbonic Anhydrase Activity

Carbonic anhydrase activity was determined as mentioned by vapoorte *et al.* [15], with the modification described by Parui *et al*. [8] using spectrophotometer. In this assay, the esterase activity of carbonic anhydrase was determined from the hydrolysis rate of 3mM p-nitrophenyl acetate to p-nitrophenol. The assay system contained

100 µL hemolysate placed in 1 cm spectrometric cell containing 1.4 ml 0.05 M Tris-HCl, pH: 7.4 and 1.5ml p-nitrophenyl acetate. The change in absorbance at 348 nm was measured over the period of 3 min before and after adding the sample. The absorbance was measured by a UV-Vis spectrophotometer (Shimadzu UV-2600 Spectrophotometer). One unit of enzyme activity was expressed as µmol of p-nitrophenol relased/min/µL from hemolysate at room temperature (25˚C) [6] [8].

2.5. Measurement of Glycated Hemoglobin (HbA1C)

Glycosylated haemoglobin determination: HbA1C was measured using standard reagent kits, according to the manufacturer's information (Spectrum-diagnostics, Egypt). The assay principle is based on a hemolysed preparation of whole blood mixed continuously for 5 minutes with a weakly binding cation-exchange resin. The labile fraction is eliminated during the hemolysate preparation and during the binding. During this mixing, HbA binds to the ion exchange resin leaving GHb free in the supernatant. After the mixing period, a filter separator is used to remove the resin from the supernatant. The percent glycosylated hemoglobin is determined by measuring absorbances at 405 nm of the ratio of the absorbances of the Glycosylated hemoglobin (Ghb) and the Total hemoglobin fraction (THb). The ratio of the absorbances of GHb and THb of the control and test is used to calculate the percent GHb of the sample.

2.6. Measurement of Biochemical Parameters

Blood glucose, Blood lactate, Blood cholesterol and Blood triglycerides were measured using (Accutrend GCT Meter, Roche, Germany with Cobas® test strips)

2.7. Statistical Analysis

Results were presented as mean ± standard Deviation (SD). Within and between groups, comparisons were performed by the analysis of variance (ANOVA) (using SPSS 20.0 for windows Computer Software Package). Significant differences were compared by Duncan's new Multiple Range test; a probability level of less than 5% ($p < 0.05$) was considered significant.

3. Results

Erythrocyte carbonic anhydrase activity was significantly higher in type II diabetic patients compared with control ($^{*}p < 0.05$). Thus type II diabetes resulted in an increase in carbonic anhydrase activity (**Figure 1**). The results showed that the Glycated hemoglobin concentration increased significantly in type II diabetic patients compared with control ($^{*}p < 0.05$, **Figure 2**).

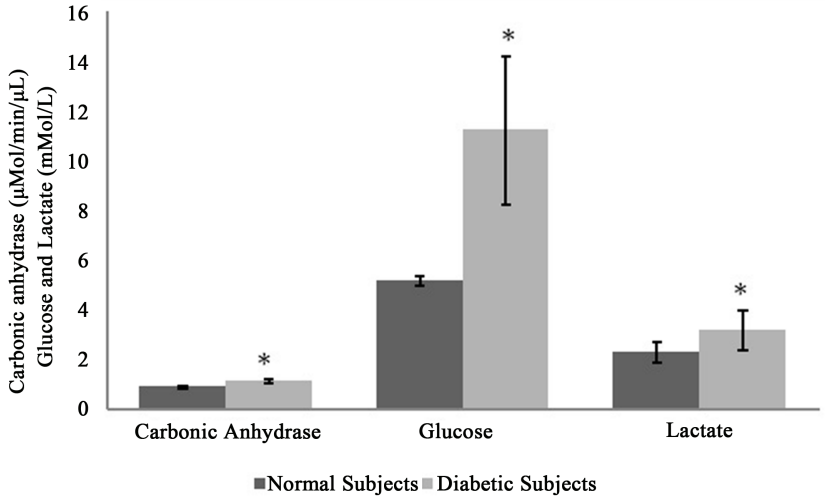

Figure 1. Erythrocyte carbonic anhydrase activity, blood glucose and blood lactate from type II diabetic patients on Metformin drug therapy. *p < 0.05 vs Normal subjects, (n = 10).

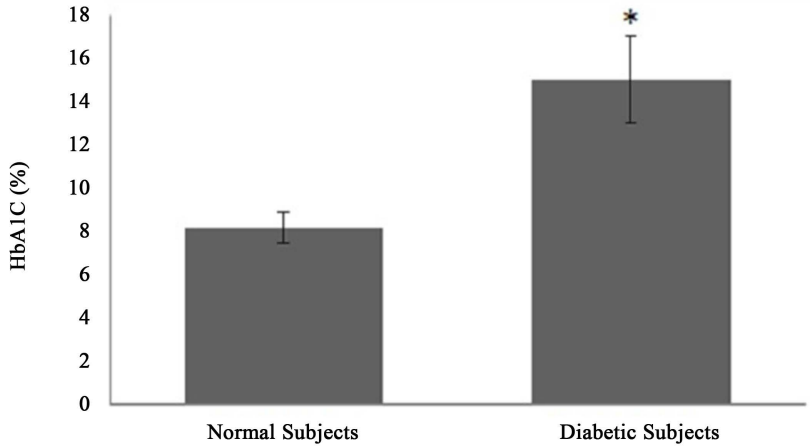

Figure 2. HbA1c (%) concentration in type II diabetic patients on metformin drug therapy. *p < 0.05 vs Normal subjects, (n = 10).

The glucose and lactate levels were significantly higher in type II diabetic patient compared to controls ($^{*}p < 0.05$, **Figure 1**). Similarly the levels of cholesterol and triglycerides were significantly increased in type II diabetic patients when compared with the controls ($^{*}p < 0.05$, **Figure 3**). Bivariate analysis showed no significant correlation between erythrocyte carbonic anhydrase with either glycated hemoglobin, glucose or with lactate in human diabetic subjects.

4. Discussion

Our data indicate that hyperglycemia results in increase in both carbonic anhydrase activity and blood lactate level. Our data suggest that the increased activity of carbonic anhydrase may be primarily the result of increased intracellular lactate level due to increased anaerobic conversion of glucose to lactate. This implies that diabetic condition that increased blood glucose concentration induces increased glycolytic rate in the erythrocytes and consequently increases the concentration of lactate which induces increases carbonic anhydrase activity. Since erythrocytes depend on the anaerobic conversion of glucose by the Embden-Meyerhof pathway for the generation of ATP. Red blood cells produce lactic acid as a byproduct of the regeneration of ATP during anaerobic glycolysis but cannot use lactic acid [1]. Lactate is transported out of the cell via monocarboxylate transporters (MCT) in an electroneutral transport mode of 1 H^+: 1 Lactate [16]. It has been reported that MCT dependent lactate-H^+ flux is facilitated by bicarbonate transporters and carbonic anhydrase (CA) activity in various cells and tissues [3] [4] [17] [18]. Our findings are consistent with recent studies in STZ induced diabetes (which is a model of type 1 diabetes) that resting blood lactate is reported to be elevated [19] [20]. However, some early evidences suggest that the changes in carbonic anhydrases activities in erythrocytes may be an initial step of altered metabolism in diabetes mellitus [6]. It has been reported that MCT1 and MCT4 transport activity is increased by interaction with carbonic anhydrase II (CAII) [4] [18] [21].

Hyperglycemia may lead to increased blood lactate accumulation, as a result of increased erythrocyte glycolytic rate for the regeneration of ATP; this in turn may lead to increased carbonic anhydrase activity to facilitate lactate out-flux into plasma to prevent intracellular lactate accumulation. Both increased lactate and increased carbonic anhydrase activity may result in decreased pH. Low pH has been reported to increase glycation of hemoglobin. Decreased pH has also been shown to reduce O_2 binding to hemoglobin (Hb) which leads to hypoxia. Hypoxia stimulates erythrocyte anaerobic ATP production, which produces lactate as the end product. Lactate must be shuttle out of the cell via the MCT's; facilitated by carbonic anhydrase to prevent intracellular lactate accumulation. Thus may also result in increased carbonic anhydrase activity.

We hypothesize that (**Figure 4**) the increased lactate efflux from erythrocyte in diabetic subjects may reflect the mass-action effect of higher intracellular lactate concentration. This mass action of lactate may account for the increased activity of carbonic anhydrase that facilitate lactate efflux from the erythrocyte of diabetic subjects into the plasma when compared to non-diabetic subjects. We may therefore report that increased gluconeogenesis in diabetic subjects may be the result of increased carbonic anhydrase activity that facilitate lactate (gluconeogenic

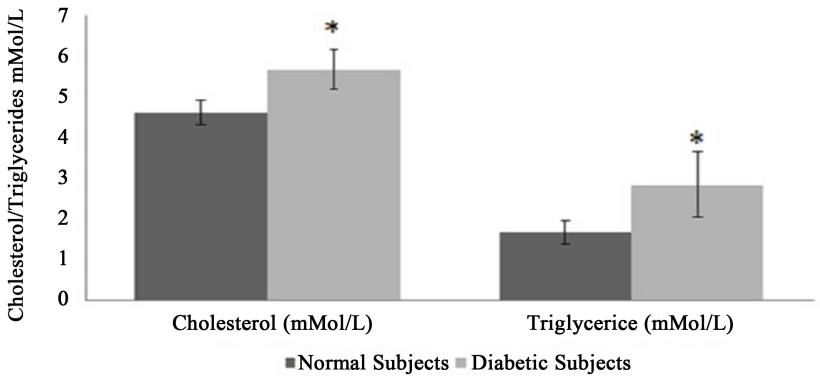

Figure 3. Blood cholesterol and triglyceride level in type II diabetic patients on metformin drug therapy. *p < 0.05 vs Normal subjects, (n = 10).

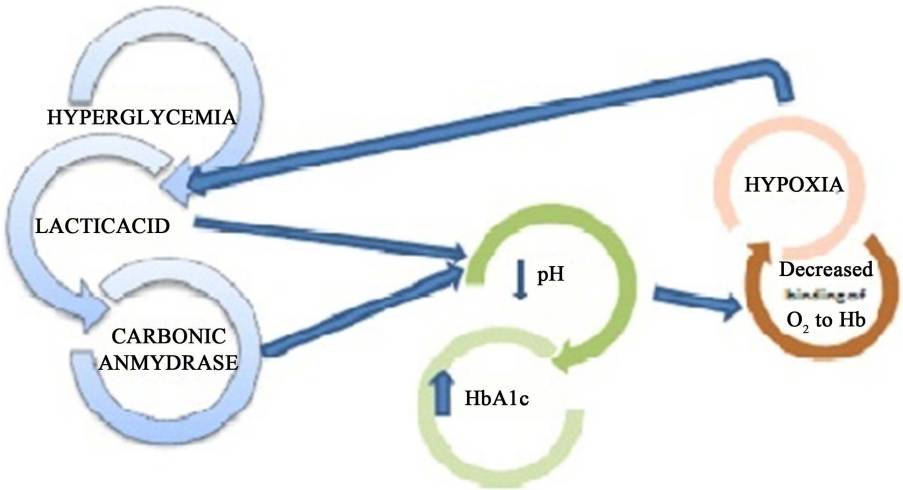

Figure 4. Hypothetical model of hyperglycemia induced carbonic anhydrase activity increase in type II diabetes mellitus.

precursor) uptake by the liver cells. Previous studies indicate that gluconeogenesis is increased in NIDDM [22] [23].

Gluconeogenesis could be increased in NIDDM because of greater delivery of gluconeogenic substrates to the liver, greater efficiency of hepatic uptake, and conversion of these substrates into glucose, or a combination of these processes. Increased rates of cori cycle have been observed in diabetic dogs [24] and have also been suggested in diabetic humans [25]. Zawadzki *et al.* [26] concluded that the rates of endogenous glucose production and the Cori cycle are increased in subjects with NIDDM.

Our results are consistent with the findings of Young *et al.* [27] who reported an association between lactate, adiposity and type 2 diabetes. In a longitudinal analysis of Swedish men, elevated serum lactate was associated with a 2.4-fold higher incidence of type 2 diabetes [28]. Kondo *et al.* [6] found that levels of both CA-I and CA-II in human erythrocytes in patients with diabetes mellitus were increased by 17.7% and 15%, respectively. They suggested that exposure of erythrocytes to high concentrations of glucose results in an increase in the concentrations of the enzymes in red cells.

Our results suggest that lactate: H^+ exiting the cell via MCTs accumulates in the plasma which leads to the dissipation of the H^+ gradient and, hence, lactic acidosis which further reduced lactate transport. This in turn may result in intracellular acidosis due to accumulation of intracellular lactate. Low intracellular pH (pHi) has been reported to increase glycation of hemoglobin as pH levels within the erythrocyte can increase (low erythrocyte pH) or decrease (high erythrocyte pH) HbA1c formation [29].

Several factors have been reported that can influence the rate of glycation of hemoglobin: pH [30]-[32], glucose

concentration (Higgins and Bunn, 1981), carbonate [33] and catalysis by 2,3-diphosphoglycerate [31] [34]-[36]. We may suggest that increased HbA1c level seen in diabetic subjects may be attributed to decreased pH_i due to decreased lactate efflux probably as a result of fall in extracellular pH due to lactate accumulation. In the present study our results showed significant increase in both total cholesterol and triglyceride level ($p < 0.05$). Diabetic dyslipidemia is associated with high plasma triglycerides, low HDL-Cholesterol and increased small dense LDL-Cholesterol particles [37]. Hypertriglyceridemia is more common in diabetics as compared to non-diabetics due to four (4) fold increase in VLDL triglyceride [38].

5. Conclusion

Diabetes may therefore be responsible for the increased activity of carbonic anhydrase, which may come as a result of increased rate of glycolysis in the erythrocyte producing higher lactate that needs to be removed to prevent intracellular lactate accumulation that may lead to drop in intracellular pHi.

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Author Contributions

All authors contributed equally to this work. Ibrahim S. Ismail carried out the studies reviewed the literature and wrote the preliminary manuscript. Ameh D. Amodu, Atawodi S. Ene-Ojo and Umar I. Alhaji supervised the project, made the final revisions and revised the preliminary manuscript, and Mohammed Fakhruddeen participated in the clinical study.

Conflict of Interests

We declare no competing interests.

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