

Artifactual Changes in Whole Blood and Plasma Glucose Levels of Diabetic and Non Diabetic Blood Samples Twenty Four Hours (24 h) Post Collection

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Authors' contributions

This work was carried out in collaboration between all authors. Author PEA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author MNE managed the literature searches and analyses of the study. Author ECN managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Analysis of diabetic blood may be delayed for hours post collection owing to several factors. Artifactual changes may occur thereby confounding the results. This study investigated some artifactual changes in glucose levels that may occur in diabetic blood stored at room temperature for 24 h. Ten (10) male Wistar rats were assigned to two (2) groups of five (5) rats per group. Group 1 rats were made diabetic by single intraperitoneal injection of 160 mg/kg of alloxan monohydrate while group 2 rats served as normal control. Rats with blood glucose values ≥ 126 mg/dl were considered diabetic. One week (7 days), following establishment of diabetes, blood samples were collected after overnight fasting from both diabetic and non diabetic rats using heparinized capillary tubes into sample bottles. Determination of the blood glucose values were

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done 1 h post collection and subsequently after every 2 h for 24 h on both whole blood and plasma. Results indicated earlier significant ($p < 0.05$) decreases in the glucose values of diabetic whole blood samples compared to the non-diabetic counterpart. Decreases in the glucose levels of whole blood sample were significantly ($p < 0.05$) higher compared to that of the plasma. It was concluded that the blood and plasma sugar levels of diabetic rats deteriorated faster compared to the non-diabetic counterpart and that significant changes in the glucose levels of both blood and plasma occurred within 2 h post collection. The plasma sugar levels of non-diabetic rats were unreliable (increased rather than decrease).

Keywords: *Artifactual changes; blood; glucose; plasma.*

1. INTRODUCTION

Diabetes mellitus is a metabolic disease condition characterized by higher than normal blood glucose values [1,2]. Diabetes mellitus type 1 results from destruction of beta cells of the islet of Langerhans which are saddled with the responsibility of producing insulin [3]. Insulin is a hormone that plays significant role in literally "dragging" blood glucose into cells [4]. In the absence of insulin consequent upon beta cell destruction, accumulation of glucose in the blood results, a condition known as hyperglycemia [4]. Diabetes mellitus type 2 is a consequence of insulin resistance [5]. It is a condition in which cells are not sensitive to insulin produced [5]. In the same vein, blood glucose accumulates.

Assessment of fasting blood glucose values is fundamental in diagnosing diabetes mellitus [6]. According to World Health Organization, demonstration of fasting blood glucose value 126 mg/dl is indicative of diabetes mellitus [7]. Fasting blood glucose levels between 100 and 125 mg/dl is considered a pre-diabetic condition [7].

Blood samples may be collected by thumb prick in humans or by tail snip as it is obtainable in laboratory animals [8]. Blood samples meant for such diagnosis may be collected in a sample bottle using an anticoagulant. If blood glucose determination does not occur immediately owing to certain bottle necks, there may be artifactual changes which could confound results [9]. There is dearth of information on possible artifactual changes that could occur in the diabetic blood glucose values.

This study was therefore designed to investigate possible artifactual changes associated with delayed determination of blood glucose. The study also aims at investigating the sensitivity and suitability of using plasma glucose rather

than whole blood glucose in diagnosing diabetes mellitus in such instances.

2. MATERIALS AND METHODS

2.1 Animals

Ten (10) adult male Wistar rats weighing between 150-200 g were obtained from the animal house facility of the faculty of Veterinary Medicine, University of Nigeria, Nsukka. The rats were acclimatized for period of 2 weeks before the commencement of the experiment. Rats were fed Vital® grower (Benin, Nigeria) feed and clean water *ad libitum*. The rats were housed in a stainless steel cage. The experimental protocol used in this study was approved by the Ethics Committee of the University of Nigeria, Nsukka and conforms with guide to the care and use of animals in research and teaching of University of Nigeria, Nsukka, Enugu state, Nigeria (ECUN/174290).

2.2 Experimental Design

Ten adult male Wistar rats were assigned into two groups of 5 rats per group. Diabetes mellitus was induced in group 1 rats while group 2 served as non-diabetic control group. On day 2 post induction, the rats came down with diabetes. One week later, fasting blood samples were collected from both the diabetic and non-diabetic groups after overnight fasting using orbital technique [8]. Blood glucose values were determined immediately upon collection using glucometer (Accu Chek Active). Each blood sample from both diabetic and non-diabetic was divided into 2 aliquots. One aliquot was centrifuged using table centrifuge at 10,000 rpm for 10 mins and the supernatant plasma collected and kept at room temperature (25°C). The plasma glucose levels were also determined using glucometer immediately upon collection. Subsequently, sugar levels of the whole blood and plasma were

determined after 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 24 h.

2.3 Induction of Experimental Diabetes Mellitus

The method of Venugopal et al. was used [10]. Induction was done by single intraperitoneal injection of 160 mg/kg of alloxan monohydrate after overnight fasting. The fasting blood glucose values were determined prior to induction. Thereafter, rats with fasting blood glucose levels of 126 mg/dl or above were considered diabetic.

2.4 Blood Sample Collection

The method of Parasuraman was used [8]. Heparinized capillary tube was inserted into the capillary plexus in the medial canthus of the eye of the rats and blood was allowed to flow into sample bottles.

2.5 Statistical Analysis

One-way Analysis of Variance was used to analyze the data on both whole blood glucose and plasma glucose values across the study duration. Variant means were separated using Duncans Multiple Range Test. Probability values equal or less than 0.05 ($p \leq 0.05$) were considered significant. Independent sample t test was used to analyze the percentage change in blood glucose of diabetic and non-diabetic blood samples. Results were presented in tables and charts.

3. RESULTS

3.1 Artfactual Changes in Blood Glucose of Diabetic and Non-diabetic Blood Samples

The diabetic blood glucose samples decreased significantly ($p < 0.05$) 1 h post collection. Thereafter, the decreases were consistent until 6 h post collection. Subsequent decreases in the blood glucose levels from the 6th hour to 12th hour post collection were not statistically significant ($p > 0.05$). However, there was a significant ($p < 0.05$) decrease 24 h post collection. There was a non-significant ($p > 0.05$) decrease in the non diabetic blood sample 1 h post collection. However, 2 h post collection, the glucose levels of non diabetic blood reduced significantly ($p < 0.05$). Thereafter, there was a

non significant change in the glucose levels until 24 h post collection (Table 1).

3.2 Artfactual Changes in Plasma Glucose of Diabetic and Non Diabetic Blood Samples

There was a significant ($p < 0.05$) decrease in the plasma glucose levels of diabetic blood 1 h post collection. Significant ($p < 0.05$) decreases were also observed 2 h, 6 h, 10 h, 12 h and 24 h post collection. The plasma glucose of non diabetic blood remained statistically the same ($p > 0.05$) 1 h post collection. However, there was a significant ($p < 0.05$) increase 2 h post collection. Such increases persisted throughout the 24 h duration of the study (Table 2).

3.3 Percentage Changes in Blood Glucose of Diabetic and Non-diabetic Blood

The percentage decrease in the blood glucose of diabetic blood was significantly ($p < 0.05$) higher than that recorded for non-diabetic blood 1 h-12 h post collection. However, there was no significant difference in the percentage decrease in the glucose levels of diabetic and non-diabetic blood 24 h post collection (Table 3).

3.4 Percentage Change in the Plasma Glucose Levels of Diabetic Blood 24 h Post Collection

There was a progressive statistically significant ($p < 0.05$) decrease in the plasma glucose values of diabetic blood samples from 1 h to 24 h post collection. However, the changes that occurred between the 6th and 8th hour were not statistically significant ($p > 0.05$) (Fig. 1).

3.5 Percentage Change in the Plasma Glucose Levels of Non-diabetic Blood 24 h Post Collection

A slight increase in the plasma glucose levels of the non-diabetic blood 4 h post collection was observed. However, 6 h post collection, a statistically significant ($p < 0.05$) sharp increase in the plasma glucose value was recorded. There was a non-significant ($p > 0.05$) change between the 6th and 10th hour post collection. Another significant ($p < 0.05$) increase was observed 12 h post collection. Plasma glucose value obtained 24 h post collection was statistically similar ($p > 0.05$) to that obtained on 12 h post collection (Fig. 2).

Table 1. Artifactual changes in blood glucose of diabetic and non diabetic blood samples

Group	Blood glucose values (mg/dl)								
	0 h	1 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
Diabetic	126.66±0.88 ^f	77.33±0.33 ^e	67.33±1.96 ^d	53.00±1.00 ^c	33.60±0.66 ^b	31.66±4.26 ^b	27.33±2.84 ^{ab}	27.33±2.66 ^{ab}	21.66±1.20 ^a
Non-diabetic	89.33±4.05 ^d	88.33±4.25 ^d	58.00±2.64 ^c	42.66±0.88 ^b	42.33±0.33 ^b	40.66±0.33 ^b	38.00±0.57 ^{ab}	41.66±1.20 ^b	30.33±4.66 ^a

Different superscripts a,b, c, d, e, f across the same row indicate significant difference at p<0.05

Table 2. Artifactual changes in plasma glucose of diabetic and non diabetic blood samples

Group	Blood glucose values (mg/dl)								
	0 h	1 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
Diabetic	144.66±0.88 ^g	112.66±5.78 ^f	87.66±1.20 ^e	85.66±2.72 ^e	74.33±1.45 ^d	77.00±1.52 ^d	63.66±1.45 ^c	53.20±2.02 ^b	26.66±1.45 ^a
Non-diabetic	91.33±0.88 ^a	90.33±0.33 ^a	93.33±0.33 ^b	94.00±0.00 ^b	98.66±0.33 ^c	99.67±0.88 ^c	99.00±0.57 ^{ab}	104.00±0.57 ^d	104.00±0.57 ^d

Different superscripts a,b, c, d, e, f, g across the same row indicate significant difference at p<0.05

Table 3. Percentage change in blood glucose of diabetic and non diabetic blood samples monitored for 24 h

Group	Blood glucose values (mg/dl)							
	1 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
Diabetic	38.66±0.37 ^b	46.66±1.76 ^b	55.33±2.33 ^b	73.00±2.52 ^b	74.66±3.38 ^b	78.00±2.51 ^b	78.00±2.00 ^b	82.33±1.20 ^b
Non diabetic	1.66±0.33 ^a	34.66±0.33 ^a	51.66±3.17 ^a	52.00±2.08 ^a	54.00±1.73 ^a	56.66±1.45 ^a	53.00±1.00 ^a	69.00±5.13 ^a

Different superscripts a,b, between the same column indicate significant difference at p<0.05

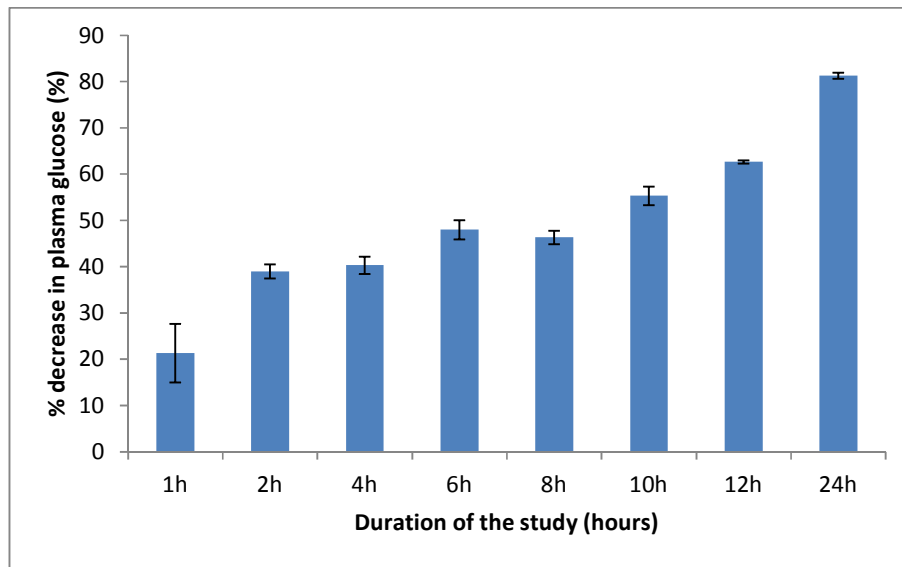


Fig. 1. Percentage decrease in plasma glucose values of diabetic blood samples monitored for 24 h

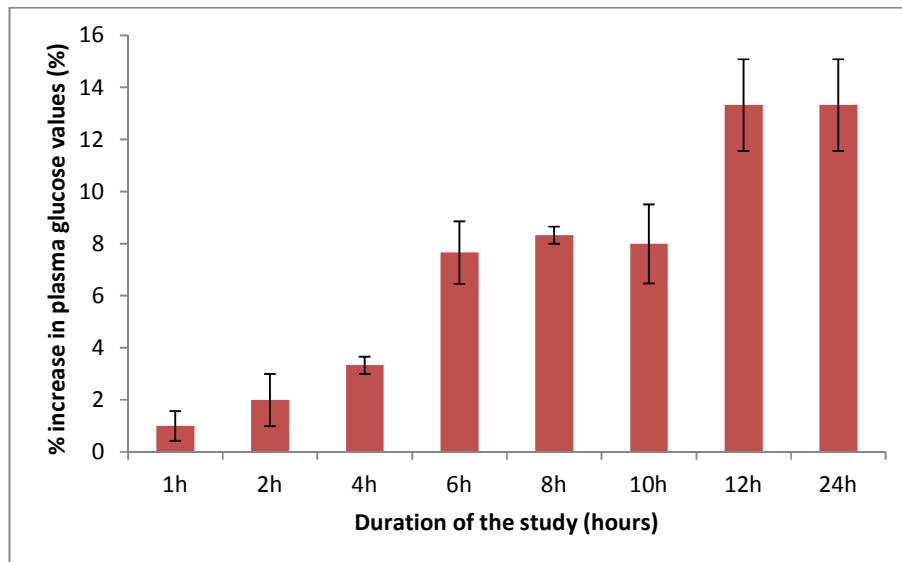


Fig. 2. Percentage change in the plasma glucose levels on non-diabetic blood samples monitored for 24 h

4. DISCUSSION

The study investigated artifactual changes that could occur in the blood glucose values of diabetic rats when kept for 24 h after collection. The consistent significant decreases in the diabetic and non diabetic blood samples observed indicated that glucose was being utilized both in the diabetic and non-diabetic blood samples. It has been reported that red blood cells can utilize glucose *in vitro* [11]. Red

blood cells utilize glucose most compared to other cells in the body but it is among the least in terms of ATP generation since glucose metabolism in the red blood cells is mainly by anaerobic process [12]. Red blood cells use glucose as their only sole source of energy [12]. Observation of more rapid reduction in the glucose values of diabetic blood when compared to that of non-diabetic blood sample is a pointer that glucose utilization in the diabetic whole blood was faster compared to the non-diabetic

counterpart. Glucose enters the red blood cell through its membrane by the help of glucose transporter protein 1 (Glut 1) [12]. Glut 1 transporters are independent of insulin in the course of transporting glucose to the cells [13]. In diabetic state, the blood circulation system is inundated with glucose which passively enters the red blood cells [14]. It is possible that the accelerated utilization of glucose in the diabetic whole blood compared to non-diabetic blood may be linked to the fact that other than anerobic glycolysis pathway, glucose is also shunted to other pathways such as polyol pathway [14]. In the diabetic state, the red blood cells are overwhelmed with glucose thus normal glycolytic pathway may not be able to metabolize the glucose thereby diverting it to other pathways such as pentose phosphate shunt [14]. However, this argument did not align with the results of studies by Driedzic et al. [15] on fish with different glucose concentrations. They reported that uptake of glucose by fish red blood cells within the species did not depend on the concentration of extracellular glucose.

There was also observation of significant decreases in the plasma glucose levels of both diabetic and non-diabetic blood. This may probably be as a result of local metabolism by the white blood cells that may have filtered into the plasma in the course of decantation. Decreases in the glucose levels were more rapid in the whole blood compared with the plasma. This was attributed to the fact that glucose utilization is a hall mark of red blood cells [11]. The observation of increases in the plasma glucose levels of non-diabetic blood samples as opposed to decreases recorded for diabetic counterpart requires further investigations to corroborate.

5. CONCLUSION

From the results of this study, the following conclusions were drawn:

- i. That much more earlier decreases in the blood glucose values of diabetic blood sample compared to that of the non-diabetic counterpart was observed
- ii. That *in vitro* deterioration of blood glucose was more rapid in whole blood compared to plasma
- iii. And that plasma glucose levels of non-diabetic blood samples increased with time rather than decrease

6. RECOMMENDATION

We recommend that sugar levels of diabetic whole blood and plasma kept under room temperature (25°C) be analyzed before 1 h upon collection while that of the non-diabetic counterpart should be analyzed before 2 h following collection.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “principles of laboratory animal care” (NIH publication No 85-23, revised 1985) were followed, as well as specific national laws. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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