

Original Research Article

THE RATE OF GLUCOSE UPTAKE BY THE CELLS: A COMPARATIVE STUDY USING DIFFERENT ANTICOAGULANTS

ABSTRACT

Background: Glucose is an important metabolic substrate in the mammalian cells, used as the body's primary source of energy. High level of plasma glucose may present with deleterious effects on several cells/tissues of the body. **Aim:** The study investigated the effect of different anticoagulants and plain container in the rate of glucose uptake by cells.

Method: Sixty-two diabetes, comprising of twenty-six males and thirty-six females attending clinic in the Federal Medical Centre, Yenagoa, Bayelsa State were recruited, and their blood samples were collected and analyzed using glucose oxidase peroxidase method, three hours thirteen minutes (3hrs:13minutes) after collection of the samples; and subsequently, repeat assays were done periodically at an interval of one hour (60minutes) for six hours (6hrs) consecutively. The data was analysed statistically and expressed as mean and standard deviation.

Results: The results demonstrated that the rate at which the blood glucose level decreased with time varied with different anticoagulants. It was observed that blood glucose in sodium fluoride oxalate, Lithium heparin, Tri-potassium ethylene Diamine-tetraacetic acid and plain containers decreased at a mean value of 0.07mmol/l, 0.12mmol/l, 0.18mmol/l and 0.35mmol/l every 60 minutes respectively. The study also showed that irrespective of the anticoagulants used, blood glucose significantly ($P<0.05$) decreased steadily showing that the concentration of blood glucose remained unstable in-vitro. Nevertheless, the rate of decrease in blood glucose in-vitro appeared to be independent in the gender (males/female).

Conclusion: From the results obtained from this study, sodium fluoride oxalate is the most appropriate anticoagulant for blood glucose estimation.

Keywords: *Glucose, Anticoagulants, Diabetes Mellitus, sodium fluoride oxalate, Lithium heparin and Tri-potassium Ethylene Diamine-tetra-acetic Acid*

1. INTRODUCTION

Glucose also known as dextrose, belongs to a group of carbohydrates referred to as simple sugars (monosaccharide): that is, they are absorbed directly into the bloodstream *via* the small intestine during digestion [1,2]. It is an important metabolic substrate that is employed as the main energy source in mammalian cells in biology [3]. The energy generated by glucose is necessary for the body's tissues and organs to function normally (including brain and red blood cell production) [2]. Glucose and other monosaccharides are transported across the intestinal wall to the hepatic portal vein and then to the hepatocytes and other tissues, where they undergo cellular respiration to be broken down or converted to fatty acids, amino acids, and glycogen or even oxidized by the various catabolic pathways of cells [4,5]. In cellular respiration, glucose and oxygen are metabolized to release energy, with carbon dioxide and water as by-products [6,4].

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from absolute or relative defects in insulin secretion, insulin action, or both [7]. There are three (3) main types of diabetes namely: Type 1 diabetes (T1DM), Type 2 diabetes (T2DM), and Gestational Diabetes (GDM) [8]. Normally, the body breaks down carbohydrate into a special simple sugar called glucose which fuels the cells in the body, but the cells need insulin in the blood stream to take in glucose and enable the cells use it for energy production. Glucose is unstable in whole blood [9]. Red blood cells possess glycolytic enzymes (enolase), hence, glucose disappears fairly rapidly due to glycolysis from whole blood. Glycolysis decreases serum glucose by approximately 5-7% in 1hour (0.3-0.6mmol/l) [10].

The proportional rate of inflow of glucose into circulation and of its utilization by body cells determines the blood glucose concentration [11]. It's level in circulation is usually subject to rigorous control by a number of homeostatic dynamics, rarely falling below 2.5mmol/L at any time nor rising above 8.0mmol/L in apparently healthy individuals after a meal or above 5.2mmol/L after an overnight fast [11]. The plasma glucose concentration is regulated by negative feedback *via* a number of hormones, primarily insulin and glucagon in order to keep the body in equilibrium [12]. The islet of Langerhans in the pancreas secretes these hormones; it is made up of alpha cells that release glucagon, beta cells that release insulin, and delta cells that release somatostatin [13]. Insulin is released into the circulation from the *beta* cells in the pancreas in response to elevated plasma glucose levels, and acts by stimulating insulin sensitive cells such as liver cells, muscle cells and fat cells to increase the uptake of glucose for metabolism [13]. However, insulin does not influence glucose uptake in the brain cells as seen in peripheral tissues, and the brain is considered insulin insensitive. The hormone glucagon has the opposite effect of insulin, and it is released into the bloodstream by the pancreatic alpha cells in response to dangerously low plasma glucose levels, and acts to increase the plasma glucose concentrations by stimulating gluconeogenesis and glycogenolysis in the liver [13].

Anticoagulants are chemical agents used to prevent the formation of blood clots in the laboratory, and are the cornerstone therapy for thrombosis prevention and treatment [14]. Generally, in the laboratory, anticoagulants are used in blood coagulation tubes or blood bags to prevent blood clots [15]. Some examples of anticoagulants commonly used in the laboratory include; heparin, fluoride oxalate, tri-sodium citrate, Ethylene diamine tetra-acetic acid and hirudin [16]. Blood is a viscous fluid that comprises of cellular elements such as red blood cells, white blood cells, and platelets suspended in the plasma. Blood collected into anticoagulant containers allow for separation into plasma which is used for the determination of several biomolecules in the blood [17]. Blood prepared without anticoagulants yields the liquid portion called serum which is used for the measurement of various chemical substances in the blood [17]. In years pasts, serum was the test sample of choice for determining the extracellular concentrations of analytes in blood. But today, plasma is preferable for most laboratory tests, though not all of them, because its constituents reflect a patient's pathological condition more accurately than serum [16]. Some alterations of constituents such as glycolysis in glucose estimation can be prevented by using anticoagulants like sodium fluoride oxalate, which retards glycolysis and preserve the level of glucose in blood sample [16].

The measurement of plasma glucose is vital for the screening, diagnosis, and monitoring of diabetes mellitus (DM). Globally, about 415 million people have diabetes; 91% of this population has Type 2 diabetes mellitus (T2 DM) [18]. Therefore, adequate monitoring of blood glucose helps to guide lifestyle interventions that would efficiently reduce the risk of becoming diabetic. This work was carried out to help medical laboratory personnel in selecting the best anticoagulant that is most appropriate for accurate plasma glucose measurement in routine laboratories as well as the turnaround time.

2. MATERIALS AND METHODS

2.1 Area of Study

This research was carried out in Federal Medical Centre, Yenegoa Bayelsa State, Nigeria. Bayelsa State has a total population of about 1,703,358 (population Census, 2006), and Yenagoa is the state capital having various industrial activities, hospitals and educational institutions. Bayelsa has eight (8) Local Government Areas namely: Brass, Ekeremor, kolokuma/opokuma, Nembe, Ogbia, Sagbama, Southern Ijaw and Yenegoa. The major occupations of the people are: fishing, farming, palm oil milling, and limbering, palm-wine tapping, Local Gin making, trading, carving and weaving.

2.2 Study Design

This work utilized analytical and comparative scrutiny. Fasting blood samples were collected from individuals attending metabolic clinic and added into plain container, sodium fluoride-oxalate, K₃EDTA and lithium heparin containers. The samples were examined at 60 minutes interval after 3hr: 13mins, 4hrs:13mins, 5hr: 13mins, 6hrs:13mins, 7hrs:13mins, 8hrs:13mins and 9hrs:13mins.

2.3 Population of Study:

A total number of sixty-two (62) patients visiting the Chemical pathology laboratory at the Federal Medical Centre Yenagoa were recruited for the study; comprising twenty-six (26) males and thirty-six (36)

females. The subjects were randomly selected from routine checkup patients within the age range of 25-60 years.

2.4 Sample Size

$$N = Z^2 \times p(1-p) / D^2$$

Where: N= maximum sample size

D =desired level of significance 0.05 (5%)

Z= confidence interval (1.96)

P= prevalence rate (4.2%) Naing *et al.* (2008)

The prevalence (4.2%) is gotten from the diabetes percentage prevalence of six (6) million people out of 140 million people from the 2006 census by Sunny, (2012).

Calculation

$$N = 1.96^2 \times 4.2\% (1-4.2) / 0.05^2$$

$$= 3.84 \times 0.042 [0.958] / 0.0025$$

$$= 0.16128 [0.958] / 0.0025$$

$$= 0.15450624 / 0.0025$$

$$= 61.80$$

$$= 62 \text{ samples.}$$

2.5 Sample Collection

After taking history and physical examination, 8ml of blood were collected from subjects by venipuncture technique and dispensed into sodium fluoride oxalate, Ethylene Diamine tetra-acetic acid (k_3 EDTA) lithium heparin, and plain container (2ml each). The samples were centrifuged at 3000rpm for 5 minutes and the plasma is obtained for glucose determination after 3 hours of sample collection. The samples were examined at 60 minutes interval, and first measurement was done at 3hrs:13mins, followed by 4hrs:13mins, 5hr: 13mins, 6hrs:13mins, 7hrs:13mins, 8hrs:13mins and 9hrs:13mins.

2.6 Estimation of Serum Glucose

The blood glucose was analyzed using Glucose oxidase-peroxidase method as described by (Trinder, 1969). Principle: In this method, the aldehyde group of β -D glucose is oxidized by glucose oxidase to give gluconic acid and hydrogen peroxide. Addition of the enzyme peroxidase and a chromogenic oxygen acceptor such as phenol and 4-aminoantipyrine results in the formation of a coloured compound [quinoimine] that can be measured spectrophotometrically at 520nm wavelength. Briefly, three test tubes were labeled as blank, standard and test respectively. 2000microlitre each of glucose working reagent was added across the three test tubes. Thereafter, 20 microlitre of distilled water, standard and sample was added to their respective test tubes. The tubes were mixed and incubated at 37°C for 10 minutes, the absorbance of the samples and standards were measured against the reagent blank at 520nm.¹⁹

2.7 Statistical Analysis

The Special Package of Social Science version 23.0 was used for the statistical analysis. The statistical significance was set at $p < 0.05$ and students t-test was used to determine the test of significance. Mean \pm SD was used to express the results.

3. RESULTS

3.1 Glucose Concentrations (mmol/L) per unit time in Different Anticoagulants

Results from Table 1 revealed that the rate at which the blood glucose decreased with time varied with specific anticoagulants. It was observed that the glucose level in the plain containers decreased at a mean value of 0.035mmol/l in every 60 minutes (1hour). The plasma glucose concentration in sodium fluoride oxalate container decreased at a mean value of 0.07mmol/l every 60 minutes (1hour). The blood glucose concentration in lithium heparin and Tri-potassium Ethylene Diamine tetra-acetic acid (EDTA) containers decreased at mean values of 0.12mmol/l and 0.18mmol/l in every 60 minutes respectively.

Table 1: Values of Glucose Concentrations (mmol/L) per unit time in Different Anticoagulants Expressed in Mean ± Standard Deviation

Anticoagulants/ Time	3hrs: 13mins	4hr: 13mins	5hrs: 13mins	6hrs: 13mins	7hrs: 13mins	8hrs: 13mins	9hrs: 13mins
Plain Container	6.56±1.14	6.24±1.18	5.96±1.11	5.59±1.14	5.36±1.11	5.09±1.05	4.76±1.03
Sodium Fluoride Oxalate Container	8.84±1.61	8.74±1.59	8.65±1.54	8.54±1.48	8.46±1.49	8.44±1.43	8.43±1.43
Lithium heparin Container	9.43±1.61	9.19±1.73	9.07±1.54	8.95±1.54	8.90±1.56	8.82±1.17	8.74±1.56
K ₃ EDTA Container	9.02±1.66	8.82±1.62	8.60±1.63	8.42±1.52	8.22±1.54	8.10±1.57	7.94±1.50

Key: The superscript * indicates $P < 0.05$ statistically significant. 3hrs:13minutes refer to the time between collection and the first estimation of the samples.

3.2 Comparison of the Glucose Concentration (mmol/l) in Different Anticoagulants and Plain Container at different Time of Measurement

Results from (table 2) showed the mean and standard deviation of glucose concentration in different anticoagulants with respect to time compared to that of plain containers. The result showed that there was a significant difference ($P < 0.05$) observed in the anticoagulant containers when compared with the plain container. Plasma glucose levels in the sodium fluoride oxalate container was significantly ($P < 0.05$) lower compared to the lithium heparin and K₃EDTA containers. However, the concentration of plasma glucose in lithium heparin container was slightly higher than that of K₃EDTA and sodium fluoride oxalate containers, but not statistically significant. Results from the study also revealed that the concentration of blood glucose decreases with an increase in the time of sample analysis in plain and anticoagulant containers.

Table 2: Comparison of the Glucose Concentration (mmol/l) in Different Anticoagulants and Plain Container at different Time of Measurement

Time/ Anticoagulants	Plain container	Sodium fluoride oxalate container	Lithium heparin container	K ₃ EDTA container	P-value
3hrs:13mins	6.56±1.14	8.84±1.61	9.43±1.61	9.02±1.	0.024*
4hrs:13mins	6.24±1.18	8.74±1.59	9.19±1.73	8.82±1.62	0.001*
5hrs: 13mins	5.96±1.11	8.65±1.54	9.07±1.54	8.60±1.63	0.000*
6hrs:13mins	5.59±1.14	8.54±1.48	8.95±1.54	8.42±1.52	0.034*
7hrs:13mins	5.36±1.11	8.46±1.49	8.90±1.56	8.22±1.54	0.000*
8hrs:13mins	5.09±1.05	8.44±1.43	8.82±1.17	8.10±1.57	0.001*
9hrs:13mins	4.76±1.03	8.43±1.43	8.74±1.56	7.94±1.50	0.000*

Key: The superscript * indicates $p < 0.05$ statistically significant; ns: not significant; 3hrs:13minutes refer to the time between collection and the first estimation of the samples.

3.3 Glucose Concentrations (mmol/L) per unit time in Different Anticoagulants with respect to Gender

Results from table 3 showed the mean values of glucose in the different anticoagulants with respect to gender (male/female). There was no significant difference observed in the blood glucose concentration between the male and female recruits.

Table 3 Values of Glucose Concentrations (mmol/L) per unit time in Different Anticoagulants with respect to Gender

Parameters	Male	Female	P-value	Remark
Plain Container	6.57±0.41	6.55±0.99	$P > 0.05$	NS
Sodium Fluoride Oxalate Container	8.86±1.60	8.84±1.60	$P > 0.05$	NS

Lithium heparin Container	9.45±1.35	9.44±1.60	P>0.05	NS
K ₃ EDTA Container	9.10±1.59	9.00±1.62	P>0.05	NS

Key: *N/S= Non significant; $p<0.05$ is considered significant. Student *t*-test was used to test the mean significant level.

4. DISCUSSION

In diagnostic medicine, it is a regular practice to analyze blood glucose in samples stored in anticoagulants. Blood samples are collected and preserved in various anticoagulants to maintain them in their natural state. However, despite being preserved in their natural state, the blood glucose levels vary depending on the anticoagulant when measured at different times. Thus, the aim of the current study is to evaluate the rate of glucose uptake by cells, as well as compare the changes in blood glucose levels over a period of nine (9) hours at intervals of one (1) hour (60 minutes), after the first run was made three hours thirteen minutes (3hrs:13 minutes) from the time of collection using the glucose oxidase peroxide method.

From the results as shown in (table 1), revealed that the rate at which the blood glucose decreased with time varied with specific anticoagulants. This is in consonance with the work done by Nwangwu et al. [20], which says that the amount of blood glucose *in-vitro* is never steady. In the present study, blood glucose concentration was observed to decrease in sodium fluoride oxalate, lithium heparin, tripotassium ethylene diamine tetra-acetic acid (k3EDTA), and plain containers at mean values of 0.07mmol/l, 0.12mmol/l, 0.18mmol/l, and 0.35mmol/l every hour (60 minutes). This finding suggests that lithium heparin tubes can replace sodium fluoride oxalate tubes and not affect diabetes mellitus diagnosis when immediately analyzing for blood glucose level. The findings also imply that blood stored with sodium fluoride oxalate as an anticoagulant tends to better maintain the glucose level over a long period of time. This might be as a result of fluoride ions' capacity to slow down glycolysis by inhibiting the enzyme enolase, which in turn slows down the formation of phosphoenol pyruvate in the glycolytic pathway. This correspond with the works of Khaled et al. [21] and Gupta and Kaur, [22] who reported the inhibitory property of fluoride oxalate on the activity of enolase. Although, Dixon and Webb, [23] have implicated lithium ion as an inhibitor of enolase, the high blood glucose values observed in the lithium heparin container could be due to heparin which is known to be a highly sulphated glycosaminoglycan. According to Nieuwdorp et al. [24], glycosaminoglycans are long, unbranched polysaccharides made of a repeating disaccharide unit comprising an amino sugar (N-acetylglucosamine or acetylgalactosamine), and N-acetylglucosamine has the chemical formula $C_8H_{15}NO_6$ and is a monosaccharide derivative of glucose. This present study is encouraged to believe this is the probable reason for the raised blood glucose levels observed in the lithium heparin containers.

The result as shown in table 2 revealed that there was a significant difference ($P<0.05$) observed in the anticoagulant containers when compared with the plain container. Plasma glucose levels in the sodium fluoride oxalate container was significantly ($P<0.05$) lower compared to the lithium heparin and K3EDTA containers. However, the concentration of plasma glucose in lithium heparin container was slightly higher than that of K₃EDTA and sodium fluoride oxalate containers, but not statistically significant. Results from the study also revealed that the concentration of blood glucose decreases with an increase in the time of sample analysis in plain and anticoagulant containers. Additionally, it was observed that blood sugar levels consistently reduced significantly ($P<0.05$) regardless of the anticoagulants used, as was also shown with k₃EDTA. This implied that anticoagulants could not entirely halt the breakdown of glucose. Therefore, over an extended length of time, the concentration of plasma glucose may reduce to zero level.

The result further revealed that there was no significant difference ($P<0.05$) observed in the glucose concentration between the male and female recruits as shown in (table 3). However, the glucose concentration in male subjects was slightly higher than the female subjects in the plain and anticoagulant

containers. This research has proved the hypothesis of sodium fluoride-oxalate being the most suitable container for glucose sample collection based on its level of glucose preservation in cells which is a practical contribution to an existing speculation in science.

5. CONCLUSION

From the findings of this study, it is obvious that irrespective of the anticoagulant used, glucose utilization by the cells is inevitable. Although, sodium fluoride oxalate has proven to be the anticoagulant of choice as it decelerates the rate of glycolysis to 0.07mmol/l in an interval of 60 minutes

RECOMMENDATION

This study therefore, recommends that glucose estimation should be carried out immediately after sample collection if reliable results are anticipated.

ETHICAL APPROVAL AND CONSENT

Ethical approval for this study was obtained from the Research and Ethical Committee of the Federal Medical Center, Yenagoa, Bayelsa State before the commencement of the work. Informed consent was taken from patients before their enrollment into the study.

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