Sedimentation by Gravity Stabilizes Plasma Glucose for Up to 60 Minutes

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ABSTRACT

Objective: Glucose levels decrease in whole blood *in vitro*, but there are several methods that minimize the loss, including special tubes and ice. This study evaluated whether sedimentation by gravity in an upright position was a viable alternative.

Design: Lithium heparinized blood was collected from 20 individuals without a diagnosis of diabetes. The samples were allowed to sediment at ambient temperature and were tested in quadruplicate at 30 minute intervals. A Repeated Measures ANOVA compared the means of each time-point.

Results: Plasma glucose results were not statistically different between 30 minutes and 60 minutes after collection ($p = 0.156$). At 90 minutes after collection, glucose was significantly different than the initial glucose readings ($p < 0.001$). Each reading thereafter also showed a statistically significant difference from the initial reading.

Conclusions: Samples for glucose measurement are stable in lithium heparin for no longer than 60 minutes when held in an upright position prior to centrifugation.

ABBREVIATIONS: GDM - Gestational Diabetes Mellitus, OGTT - Oral glucose tolerance test

INDEX TERMS: lithium heparin, plasma glucose, glycolysis, sedimentation

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INTRODUCTION

It is well established that glucose levels decrease in whole blood *in vitro*, with estimates that the rate of glycolysis is 5-7% per hour.[1] The most effective method to minimize glycolysis is the immediate separation of blood cells from plasma or serum.[2] The use of NaF to inhibit glycolysis is widely used but is imperfect. Glucose continues to decrease in NaF treated blood during the first four hours after collection, albeit at a slower rate than blood collected in heparin.[3] Acidification of fluoridated blood by citric acid buffer further inhibits glycolysis.[4] The American Diabetes Association position statement on glucose collection recommends three possible treatments to minimize the effect of glycolysis, that is 1) immediate separation of cells from plasma, 2) placing the tube in an ice slurry with separation of plasma from cells within 30 minutes, or 3) where available, the sample may be collected in a tube with citrate buffer and NaF.[5] Solution 1 is difficult to apply, and solutions 2 and 3 minimize the utility of the plasma for other analytes; ice slurry can lead to an alteration in potassium measurement,[6] and the acidified NaF tube is limited to glucose measurement.

The diagnostic threshold for diabetes is a glucose of 126 mg/dL (7.0 mmol/L) or greater for fasting glucose and/or 200 mg/dL (11.0 mmol/L) or greater for a 2 hour glucose tolerance test. The diagnosis of Gestational Diabetes Mellitus (GDM) can be made by a fasting glucose ≥ 92 mg/dL (5.1 mmol/L), or, following a 75 g glucose load, a 1 hour glucose ≥ 180 mg/dL (10.0 mmol/L) or a 2 hour glucose ≥ 153 mg/dL (8.5

mmol/L).[7] Patients with a glucose value close to the diagnostic threshold may receive an erroneous interpretation if the sample is not processed soon after collection, even if the samples have been treated with sodium fluoride.

The recommendation to inhibit glycolysis by submersion in an ice slurry is the least expensive option, but it is inconvenient. We believe it will be met with opposition from those collecting blood, and applied haphazardly. Our experience in blood collection and processing led us to ask the question whether sedimentation of cells from plasma produced by gravity on whole blood samples collected in lithium heparin and held upright would provide the same effect on inhibiting glycolysis as that of centrifugation. To that end, we collected whole blood samples in lithium heparin from a random sample of individuals without a diagnosis of diabetes, and allowed the samples to sediment by gravity for intervals of time up to and including 240 minutes. At 30 minute endpoints we centrifuged an aliquot and tested the plasma glucose.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board at the University of West Florida, and all participants signed informed consent.

Subjects

Twenty men and women between the ages of 22 to 66 who had not been diagnosed with diabetes mellitus were recruited from the University of West Florida.

Laboratory analysis

Non-fasting blood was collected in Lithium Heparin tubes (BD), mixed well by inversion and transported to the testing laboratory. No special treatment was used during the transport time. Transport was complete within 30 minutes, and sample processing began at precisely 30 minutes post collection. Samples were well mixed, and aliquotted as 500 - 600 microliters into 1.5 ml microcentrifuge tubes and held upright at room temperature (24ºC). At 30 minute intervals each successive sample was mixed briefly by inversion, and then immediately centrifuged at 1000 RCF for 5 minutes. The plasma was transferred and stored frozen at -20ºC for no longer than one month. Frozen plasma samples were thawed at room temperature, vortexed briefly, and tested in quadruplicate by hexokinase methodology on a Vista 1500 Chemistry analyzer (Siemens) at West Florida Hospital, Pensacola, Florida. Imprecision of the method was 2.7% at normal values (81 mg/dL/ 4.5 mmol/L) and 2.3% at high values (280 mg/dL/ 15.6 mmol/L). Results shown for glucose are the average of the four replicates.

Statistical analysis using SPSS Statistics 19 (IBM)

A Repeated Measures ANOVA was performed on the glucose data using a 95% Confidence Level. Mauchly's test indicated that the assumption of sphericity had been violated, χ^2 (27) = 74.991, therefore degrees of freedom were corrected using Greenhouse-Geiseer estimates of sphericity (ε= 0.371). Post Hoc tests used Bonferroni Correction.

RESULTS

Twenty male and female adults participated in the study. Table 1 shows their characteristics. Each glucose time-point was tested in quadruplicate and the average value for each is shown in Table 2. Subject 8 and 18 had insufficient sample for one of the time-points, so they were excluded from further analysis (n=18).

Table 1. Characteristics of participants

	n	Age in years*	% Female	%non-Hispanic Caucasian
Subjects	20	37.4 (15.1)	50	80

*Values are mean (SD)

Figure 1 shows the mean glucose level of all 18 subjects, averaged for each time-point. The average ranges from 94.54 mg/dL (time=30 minutes) to 83.28 mg/dL (time=240 minutes). The results show that the mean glucose levels are significantly affected by the time elapsed from collection, ($F(2.600,44.192)=82.904$, $p<0.001$, partial η^2 = 0.830. Further analysis shows there is no statistically significant time*sex interaction effect ($F(2.785,44.568)=2.276$, $p>0.05$). Figure 2 shows the mean glucose for all subjects at specific time-points independently for men and women. This analysis demonstrates there is no intersection between the mean glucose plots of male and female, further indicating no interaction. Therefore, the sex of the patient does not cause an effect on the decrease of the plasma glucose.

Post Hoc tests using Bonferroni Correction revealed that there was a slight difference in plasma glucose

readings at 30 minutes and 60, but this was not statistically significant (p=0.156). However, after 90 minutes of elapsed time, the average plasma glucose level was significantly different than the initial glucose readings (p<0.001). Each reading thereafter (measurements taken at 120, 150, 180, 210 and 240 minutes) also showed a strong statistically significant difference from the initial reading of the subject's plasma glucose. Therefore, it can be concluded that plasma glucose readings are stable only up to 60 minutes when the lithium heparin tube is kept at ambient temperature in an upright position.

Table 2. Laboratory analysis. Glucose results displayed in minutes after collection

ID Code	Glucose** in mg/dL at 30 minute time-points							
	30	60	90	120	150	180	210	240
1	90.8	89.8	84.0	85.3	83.3	81.8	79.3	79.3
2	83.3	81.5	79.3	80.5	77.8	77.8	74.3	74.3
3	100.5	96.8	96.0	95.0	93.3	92.0	90.3	91.8
4	80.5	77.5	77.3	75.3	74.3	70.3	70.3	68.5
5	72.5	68.3	66.3	61.0	59.5	56.0	55.0	51.3
6	82.8	81.8	78.3	76.8	74.5	74.0	72.0	70.0
7	121.8	121.0	119.3	121.5	117.3	116.8	117.0	116.5
8	77.5	77.8	74.3	72.0	69.0	NR*	67.3	63.0
9	78.3	73.3	72.8	69.5	69.5	67.3	67.5	63.5
10	115.8	110.3	111.5	109.3	107.0	102.0	105.0	98.8
11	62.8	61.5	60.8	58.0	56.5	54.8	53.8	50.8
12	89.5	88.0	87.5	87.0	86.5	86.0	83.8	82.5
13	86.5	85.0	81.0	80.5	77.5	76.3	74.3	74.3
14	79.8	77.5	73.8	72.8	72.5	69.5	67.0	69.5
15	97.8	93.5	94.3	89.5	87.5	84.5	81.5	83.3
16	199.3	203.5	201.0	195.0	199.5	198.7	192.8	196.5
17	92.8	88.8	88.8	88.3	86.8	85.0	86.0	84.8
18	83.5	83.5	80.5	80.3	NR*	76.8	75.5	72.0
19	86.5	84.0	80.5	78.3	75.0	73.8	73.5	70.5
20	81.0	84.8	83.3	79.5	78.0	76.8	73.5	73.3

*NR indicates no result obtained; **Glucose performed in quadruplicate and shown as the average. Conversion factor to mmol/L for glucose = mg/dL ÷18.

DISCUSSION

We have demonstrated here that collecting whole blood in lithium heparin, placing it in a rack, and allowing it to sediment by gravity at ambient temperature will significantly delay glycolysis for up to 1 hour, but not beyond that. We show here that the rate of glycolysis after 60 minutes, while slower than what has been reported in the literature[1] produces a statistically significant difference in glucose value. In the clinical laboratory, sample volumes vary widely. A blood sample for glucose may arrive with a volume from 600 microliters to 10 milliliters, depending on the patient size and accessibility, the laboratory choice of tubes, and the fullness of the tube. Plasma glucose is lowered *in vitro* by the effect of erythrocytes and leukocytes on the blood. We expect that a smaller volume of blood will show a greater loss of glucose than a larger volume, since the volume of plasma relative to the cellular interface (buffy coat) is smaller. By using small volume aliquots, we have tested the maximum limit of this effect.

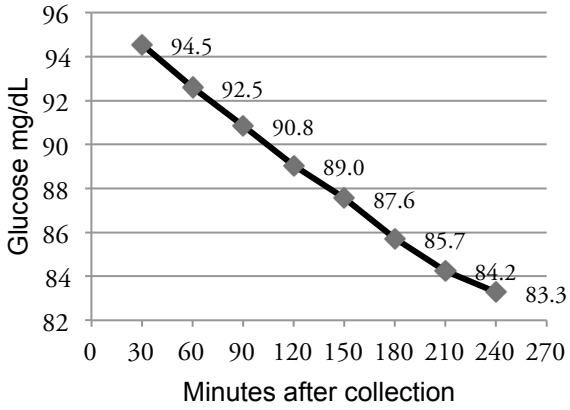

Figure 1. Mean decline of blood glucose from 30 minutes to 240 minutes in study subjects

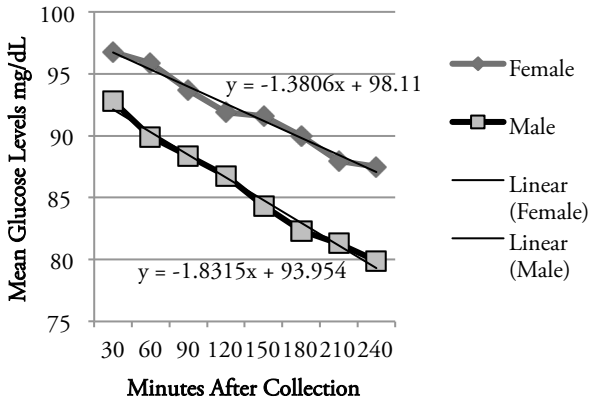

Figure 2. Mean glucose levels for all subjects at 30 minute increments separated by sex

Plasma glucose is measured for diagnosis of diabetes as well as for monitoring the glycemic status of patients with known diabetes who are hospitalized. Glucose is also part of routine chemical panels, which may be

ordered for an annual physical. A delay of one hour in sample processing is least likely to occur in a hospital setting. In-patient and emergency patient samples are delivered to laboratories promptly, and turn-around times are closely monitored by laboratory information systems. Our results indicate that it is not necessary to ice the sample during this time, but instead to keep it upright in a rack during transport. Outpatient collection facilities are also likely to benefit from this finding, as they can take advantage of the 60 minute window to maximize workflow and to implement batch methods to process samples.

Our finding that plasma glucose differs after 60 minutes even when the tube is held upright is a convincing argument to establish a protocol for collection, storage and transport of blood samples for glucose. This is especially relevant when testing for GDM in pregnant women. The 75-g 2-hour glucose tolerance test (OGTT) consists of a fasting glucose, and a 1 and 2 hour measurement. Each time-point has its own diagnostic cutoff. Unlike the diagnosis for diabetes mellitus, the diagnosis for GDM does not require confirmation by a repeat sample collected on a separate day. From our practical experience, we report that it is not uncommon for 2 or 3 of the OGTT tubes to be delivered to the lab simultaneously for processing. Based on the results of this study, batch processing of OGTT samples has a deleterious effect on the fasting and 1 hour glucose result. Similarly, the use of NaF tubes alone for OGTT should be discouraged, since the plasma glucose in NaF does not become stable until four hours has elapsed.[4] When separation of the cells from the plasma cannot be accomplished within 1 hour of collection, the specimen should be collected in NaF tubes augmented with citric acid, or NaF tubes placed into an ice slurry and processed as soon as possible.

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REFERENCES

1. Bruns DE, Knowler WC. Stabilization of glucose in blood samples: why it matters. Clin Chem 2009;55:850-2.
2. Shi RZ, Seele ES, Bowen R, Faix JD. Rapid blood separation is superior to fluoride for preventing in vitro reductions in measured blood glucose concentration. J Clin Pathol 2009;62:752-3.
3. Chan AYW, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem 1989;35:315-7.
4. Gambino R, Piscitelli J, Ackattupathil TA, Theriault JL, Andrin RD, Sanfilippo ML, et al. Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. Clin Chem 2009;55:1019-21.
5. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care 2011;34:e61-e99.
6. Lin YL, Smith CH, Dietzler DN. Stabilization of blood glucose by cooling with ice: an effective procedure for preservation of samples from adults and newborns. Clin Chem 1976;22:2031-3.
7. ADA. Standards of medical care in diabetes – 2012. Diabetes Care 2012;35:S11-S63.