Overestimation of Hypoglycemia in Infants with a High Hematocrit

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Background: In neonates, hypoglycemia is an emergency condition requiring urgent treatment. Therefore, rapid and reliable blood glucose measurements are necessary. However, this step has been proven difficult because of both analytical and preanalytical variables. In our children’s hospital, we incidentally observed cases of hypoglycemia that were not in line with the clinical picture of the infants. Remarkably, most of these infants had a high hematocrit.

Methods: Glucose concentrations were determined in blood samples from healthy participants that were collected in Li-heparin capillary and pediatric tubes. The effect of hematocrit on glucose consumption over time was studied by artificially increasing sample hematocrits. To study the effect of sample cooling, glucose concentrations were followed over time in samples stored at room temperature and on ice.

Results: In all samples, glucose concentrations declined with time. This effect was most dramatic [up to 18 mg/dL (1 mmol/L) in the first 30 min] in samples with high hematocrits and collected in capillary tubes. Cooling of samples clearly reduced glucose consumption; however, this was not evident in the first 30 min.

Conclusions: Overestimation of hypoglycemia in infants must be considered if samples are not centrifuged or are not analyzed immediately after sampling. The extent of overestimation is more pronounced in samples with a high hematocrit, collected in capillary tubes. Cooling of samples does not prevent glucose consumption in vitro during the first 30 min. These results emphasize that, for glucose analysis, prompt handling of samples of newborns with a high hematocrit is necessary.

IMPACT STATEMENT

Neonates and other patients with a high hematocrit will benefit from the information presented here. Evidence presented on preanalytical aspects of glucose measurements will help to prevent falsely decreased plasma glucose levels. Knowledge in the field of preanalytical aspects of glucose measurements and correct interpretation of results will be advanced by the information presented.

Hypoglycemia is a common finding in newborns and is associated with a wide variety of disorders, but is clinically difficult to detect (1, 2). In Wilhelmina Children’s Hospital (University Medical Center Utrecht), plasma glucose levels are determined using a Rapidlab 865 blood gas analyzer.
(Siemens Diagnostics) enabling rapid and precise analysis in a small volume (3). However, incidentally, we observed cases of hypoglycemia that differed from earlier laboratory results or were not consistent with the clinical condition. Remarkably, most infants with a suspected falsely low plasma glucose had a high hematocrit level. Although the blood gas analyzer uses whole blood as the sample type, glucose is measured in plasma. Therefore, the well-described decrease in glucose concentrations in lysed whole blood samples with a high hematocrit, due to the volume expansion by membrane lipids and proteins, cannot explain the unexpected low glucose concentrations (4, 5). The most likely explanation is increased glucose consumption by the high number of erythrocytes. In addition, we observed that unexpectedly low glucose levels were found more often in capillary tube specimens than in pediatric tubes. To investigate these observations, we studied the temporal glucose consumption in samples having different hematocrits collected in both capillary tubes and pediatric tubes.

METHODS

Effect of hematocrit on glucose consumption in vitro

Blood samples from 8 healthy participants were collected in 5-mL lithium heparin tubes (BD Diagnostics), the glucose concentrations were determined ($t = 0$), and samples were divided into 2 aliquots and centrifuged for 10 min at 1500g. Subsequently, the hematocrit of each second sample was increased by removing some of the plasma before remixing the cells and plasma. Hematocrits were determined on a Cell-Dyn 4000 hemocytometry analyzer (Abbott Laboratories). Subsequently, at 15, 30, 60, 90, 120, and 180 min, glucose concentrations in both aliquots samples were determined.

Effect of sample tube on glucose consumption in vitro

To compare temporal glucose consumption in blood collected in capillary vs pediatric tubes, blood samples from 8 healthy participants were collected in 5-mL lithium heparin tubes and centrifuged for 10 min at 1500g. Subsequently, hematocrits were increased by removing plasma. After remixing cells and plasma, glucose concentrations were determined ($t = 0$). Hereafter, 6 MultiCap™ heparinized glass heparin capillary tubes (Siemens Diagnostics) and 6 pediatric heparin tubes (Terumo Capiject System; Omnilabo) were filled from each primary tube. Capillary tubes were placed horizontally on a table, and pediatric tubes were placed vertically in racks. At 15, 30, 60, 90, 120, and 180 min, glucose levels were determined.

Effect of temperature on glucose consumption in vitro

To study the effect of temperature on temporal glucose consumption, 8 blood samples were collected in 5-mL lithium heparin tubes, centrifuged for 10 min at 1500g, and hematocrits were increased by removing plasma. After remixing the cells and plasma, the glucose concentration was determined ($t = 0$ min). Subsequently, 12 capillary tubes were filled from each primary tube. Six capillary tubes were placed horizontally at room temperature, and 6 capillaries were placed horizontally on ice. At 15, 30, 60, 90, 120, and 180 min, glucose levels were determined.

RESULTS

Effect of hematocrit on glucose consumption

All samples showed a clear decrease in glucose concentration over time. The decrease was higher in the samples with an artificially increased hematocrit. In the original samples with hematocrits <0.5, the mean decrease in glucose concentration in the
first 15 min was 4.1 mg/dL (0.23 mmol/L) (SD 4.7, 0.26) and 6.8 mg/dL (0.38 mmol/L) (SD 5.4, 0.30) after 30 min. In samples with artificially increased hematomcrits (≥0.5), the mean decrease in glucose concentration in the first 15 min was 13.1 mg/dL (0.73 mmol/L) (SD 5.9, 0.33) and 15.8 mg/dL (0.88 mmol/L) (SD 6.3, 0.35) after 30 min. Results of a representative pair of duplicate samples are shown in Fig. 1, demonstrating a decrease in glucose concentration over time that was more dramatic in the duplicate samples with increased hematocrits.

**Effect of sample tube on glucose consumption in vitro**

To study the effect of sample tube type, temporal glucose consumption was studied in both pediatric tubes and capillary tubes. The mean decrease in glucose concentration in the first 15 min was 13.1 mg/dL (0.73 mmol/L) (SD 5.9, 0.33) in capillary tubes and 0.5 mg/dL (0.03 mmol/L) (SD 1.8, 0.10) in pediatric tubes. After 30 min, the mean decrease was 16.2 mg/dL (0.90 mmol/L) (SD 4.3, 0.24) in capillary tubes and 4.1 mg/dL (0.23 mmol/L) (SD 4.7, 0.26) in pediatric tubes. Both in capillary tubes and in pediatric tubes, there was a clear decrease in glucose concentration over time. The effect was more pronounced in capillary tubes than in pediatric tubes, evidently due to the large exchange surface between erythrocytes and plasma in horizontally stored capillaries. Results of a representative pair of pediatric tubes and capillaries (hematocrit 0.73) are shown in Fig. 2.

**Effect of temperature on glucose consumption in vitro**

The effect of storage temperature was studied by comparing glucose consumption over time in blood collected in capillary tubes stored on ice and at room temperature. Although there was a clear effect of cooling on temporal glucose concentrations, this effect was not evident in the first 30 min. Results of a representative pair of capillaries are shown in Fig. 3.

**DISCUSSION**

In this study, we show that overestimation of hypoglycemia in infants has to be considered if samples are not centrifuged or analyzed immediately after sampling. This effect is more pronounced in samples with a high hematocrit and collected in capillary tubes.
Accurate measurement of blood glucose has proven difficult because of preanalytical variables (6). In the past, both lysed whole blood and plasma samples have been used for glucose measurements. Whole blood glucose levels are approximately 10%–15% lower than plasma levels, due to volume expansion by membrane lipids and proteins after lysis of blood cells. Most laboratories have calibrated whole blood glucose results to plasma levels resulting in comparable results in patients with healthy hematocrits. However, in patients with increased hematocrits, plasma calibrated whole blood glucose measurements still lead to falsely decreased glucose levels due to extra volume expansion after lysis of blood cells. In addition, sample type and method of sampling can introduce variation. During fasting, venous and capillary glucose levels are comparable to arterial glucose levels. However, arterial levels can be as much as 25.2 mg/dL (1.4 mmol/L) higher 60 min after an oral glucose load (7, 8). Finally, ongoing glycolysis can occur in blood cells in vitro if samples are not processed or analyzed directly after sampling. This step can lead to falsely decreased glucose levels (9). Sacks et al. (10) described that the decrease of glucose concentration in vitro is acceptable if samples are centrifuged or analyzed within 1 hour. These results are in contrast with our results showing that even when samples are analyzed within 30 min, a decrease in glucose concentration up to 18.0 mg/dL (1 mmol/L) is possible in samples with high hematocrit. This in vitro glycolysis can be inhibited by use of sodium fluoride or by placing samples on ice. Unfortunately, sodium fluoride does not inhibit the glycolysis efficiently in the first hour after sampling (11). Here we show that storage on ice does not prevent consumption of glucose in the first 30 min. In addition, storage on ice can lead to falsely increased potassium levels determined in the same samples (12). Recently, tubes containing a combination of fluoride and citrate have been described that are effective at preventing glucose consumption in vitro (13, 14). However, these devices are not yet available as capillary or pediatric tubes.

The best option to prevent falsely decreased glucose concentrations due to glucose consumption in vitro is direct analysis after sampling. Point-of-care (POC)2 glucose devices have been introduced. This mitigates the delay in analysis after sampling; however, this strategy does not solve all preanalytical and analytical issues. Most POC devices measure glucose in whole blood but are calibrated using plasma. Therefore, negative bias can still occur in patients with a high hematocrit due to volume expansion. Correlation of POC devices with central laboratory analyzers is often done with samples that have been analyzed immediately on both, bypassing preanalytical variables such as time to analysis, sample origin, and specimen type. Several studies have shown good correlation (15–17), but some authors report that results from specific patient groups did not show acceptable correlation (18, 19). In addition, it is suggested that, at present, no POC method is sufficiently reliable and accurate in the low range to allow usage as the sole method for screening neonatal...
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hypoglycemia (20). In addition, studies in neonates are limited because of small sample volumes. In our study, we therefore used samples from healthy participants with artificially increased hematocrits designed to mimic neonatal blood samples.

In conclusion, falsely low glucose results in infants must be considered if samples are not centrifuged or analyzed immediately after sampling.

The in vitro decrease in glucose concentration is difficult to predict but is clearly more pronounced in samples with a high hematocrit and collected in capillary tubes. Prompt centrifugation or analysis after sampling is the only solution to this problem. In our hospital, we have chosen to introduce cartridge glucose POC testing in neonatal areas to avoid overestimation of hypoglycemia due to glucose consumption by blood cells in vitro.

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REFERENCES

