ORIGINAL PAPERS

MEASUREMENT OF PLASMA GLUCOSE UNDER HYPERBARIC OXYGEN CONDITIONS

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Key Words

Equipment, hyperbaric oxygen, hyperbaric research, plasma glucose measurement.

Abstract

This study was designed to evaluate the accuracy of the bedside glucometer SureStepPro (SSP, LifeScan Inc; Milpitas, California) against a standard laboratory instrument (Yellow Springs Instruments [YSI] 2300 STAT PLUS; Yellow Springs, Ohio) during hyperbaric oxygen therapy. Human blood samples were used to prepare plasma glucose (PG) concentrations over a range 25–300 mg/dl (1.4-16.6 mmol/l). Samples were sequentially tonometered (Instruments Lexington [IL] 237 Lexington, Maryland) with two separate gas mixes at 203 kPa (2 bar) to PO_2 values of 159 kPa (1,200 mmHg, 1.6 bar) and then 8.1 kPa (60 mmHg, 0.08 bar), allowing measurement of each blood sample at both PO_2 values. PG concentrations were immediately measured by a SSP at 203 kPa (2 bar) then analysed outside the chamber by both instruments. The YSI PG measurements were unaffected by high PO_2. Compared with PG concentrations measured at PO_2 of 8.1 kPa (60 mmHg, 0.08 bar), the YSI readings at PO_2 of 159 kPa (1,200 mmHg, 1.6 bar) were higher by only 1.8 ± 1.6 mg/dl (0.1 ± 0.09 mmol/l). At PO_2 8.1 kPa (60 mmHg, 0.08 bar), compared with the YSI, the mean bias and imprecision (SD of bias) of the SSP were 6.8 and 5.7 mg/dl (0.38 and 0.32 mmol/l). At PO_2 159 kPa (1,200 mmHg, 1.6 bar), the bias and imprecision of the SSP were 4.6 and 4.8 mg/dl (0.26 and 0.27 mmol/l). The PG concentrations at PO_2 159 kPa (1,200 mmHg, 1.6 bar) measured by the SSP, when used inside the hyperbaric chamber at 203 kPa (2 bar) were lower by only 3.5 ± 4.5 mg/dl (0.19 ± 0.25 mmol/l) compared with SSP values at 101 kPa (1 bar). Therefore, the SSP provides accurate measurement of PG in blood when used either at 101 kPa (1 bar) or inside the hyperbaric chamber at 203 kPa (2 bar).

Introduction

Diabetic patients make up a significant proportion of those treated with hyperbaric oxygen (HBO_2). These patients are at risk of a decrease in plasma glucose (PG) following HBO_2. Springer reported that glucose levels decreased an average of 51 mg/dl (2.8 mmol/dl) in 25 insulin-dependent diabetic patients after several HBO_2 treatments.[1] Hypoglycaemia during HBO_2 may cause the patient to exhibit seizure activity mimicking the neurological manifestations of oxygen toxicity. Because the treatment for hypoglycaemia differs from that of O_2 toxicity, the need to measure PG in patients during HBO_2 therapy becomes a necessity.

To date, investigations and clinical management of this phenomenon are confounded by erroneously high or low glucose measurements during HBO_2.[2-4] These measurement errors have also been described at high altitude and are believed due to either an instrument artifact induced by PO_2 variation or pressure induced instrument malfunction.[5-7] The standard glucose testing system uses a glucose-oxidase reaction shown in Figure 1, in which glucose oxidase (GO) impregnated reagent strips catalyse the reaction between glucose and oxygen (O_2) to form gluconic acid and hydrogen peroxide (H_2O_2). The hydrogen peroxide then reacts with a chromogen to form a coloured compound, which is monitored by reflectance photometry.

There are a number of additional limitations with previous studies. Either they have not reported the elevated PO_2 value[2-4] or failed to reference their results to a laboratory standard instrument.[2,3] Furthermore, the highest PO_2 tested was approximately 78 kPa (590 mmHg, 0.77 bar) in a study in which Edge et al.[4] exposed glucometers to air at 375 kPa (3.7 bar). Finally, no one has yet determined whether laboratory instruments are accurate at PO_2 values achieved during HBO_2.

Since the glucose oxidase reaction depends on PO_2, it is conceivable that extremely high PO_2 may either accelerate the reaction or change the equilibrium at reaction completion, resulting in a falsely elevated blood glucose measurement. Older test strip format design may make access for oxygen difficult. These strips have been

GLUCOSE + O_2 + *GLUCOSE OXIDASE* TO GLUCONIC ACID + H_2O_2

H_2O_2 + Reduced Chromogen (UNCOLOURED) to H_2O + Oxidised Chromogen (COLOURED)

Figure 1. The Glucose Oxidase reaction.

designed to function under relative low PO_2 conditions. The oxygen content in the sample may compete with the dye (reactant) in the oxidation reaction creating an oxygen dependency, resulting in a falsely low glucose measurement.

In summary, the effect of hyperbaric oxygen on blood glucose measurements has not been adequately addressed. Our study was designed to compare the measured PG in a blood sample at PO_2 values of 8.1 kPa (60 mmHg, 0.08 bar) and 159 kPa (1,200 mmHg, 1.6 bar) using a standard laboratory instrument (Yellow Springs Instrument [YSI] 2300 STAT PLUS; Yellow Springs, Ohio) to PG measured by a bedside glucometer (SureStepPro [SSP], LifeScan Inc; Milpitas, California) over the range of atmospheric and oxygen pressures encountered in clinical HBO_2 therapy.

Methods

BLOOD SAMPLES

PG concentrations were prepared over the range 25-300 mg/dl (1.4-16.6 mmol/l). Low values were achieved by incubation for 3 to 5 hours at 37°C in a water bath with constant rotation to the sample tubes. A curve of the decrease with time of PG in a blood collection tube (Vacutainer TM 5 ml; Becton Dickinson Inc; Franklin Lakes, New Jersey) containing lithium heparin (5U/ml) enabled appropriate timing to achieve low PG concentrations. High values were obtained by adding 10 to 30 µl of 50% dextrose to sample tubes.

In keeping with common clinical practice, we initially measured PG from the same blood sample in both LiHep (lithium heparin) and NaF (sodium fluoride) tubes. Fluoride prevents glycolysis by inhibiting glucose oxidase thus providing stable PG concentrations in blood collection tubes. The YSI was unaffected at 250 mg/dl NaF, as expected.[8] Unfortunately, the SSP was greatly affected by this concentration of NaF (see results). Therefore, all blood glucose measurements for the study were on samples containing lithium heparin.

INSTRUMENTS

The four instruments studied were a SSP glucometer, placed inside the hyperbaric chamber, and YSI, SSP, and IL instruments (Instruments Lexington [IL] 1640, Lexington, Maryland; blood gas analyser) located just outside the hyperbaric chamber. Each was calibrated according to the manufacturers' protocols. Immediately prior to tonometry, the three glucose instruments measured a baseline glucose concentration for each sample.

The 5 ml venous blood samples were emptied into the tonometer (IL 237, Lexington, Maryland) maintained at 37°C and the chamber was then pressurised to 203 kPa (2 bar). The tonometered blood sample PO_2 was typically stable at 159 kPa (1,200 mmHg, 1.6 bar) after an hour at 203 kPa (2 bar) using a gas mixture containing 94% O_2, 3% CO_2 and 3% N_2. Confirmation of PO_2 values were by the IL 1640 immediately after sample decompression. Although blood gas analysis was at 101 kPa (1 bar), not 203 kPa (2 bar) this method of measurement has been shown to be clinically accurate.[9] At a PO_2 159 kPa (1,200 mmHg, 1.6 bar) a gas tight glass syringe was used to withdraw half the blood sample, which underwent immediate PG measurement by the SSP in the chamber. The syringe was then decompressed over 5 seconds through a medical lock to 101 kPa (1 bar). Within 2 minutes this syringe sample had blood gas analysis and PG measurement by both the laboratory instrument and bedside glucometer.

Interim analysis of the first 13 samples showed a significant drop in PG as a result of tonometry to PO_2 159 kPa (1,200 mmHg, 1.6 bar), presumably as a result of ongoing glucose consumption by leucocytes and erythrocytes in the sample. Therefore, in order to measure glucose on a blood sample at both high and low PO_2 values within a short time interval, the following procedure was performed on 21 samples. After aspiration of a blood sample from the tonometer at a PO_2 of 203 kPa (2 bar), the remaining blood was tonometered at 203 kPa (2 bar) with a gas mixture containing 94% N_2, 3% O_2, 3% CO_2. Serial PO_2 measurement confirmed that a PO_2 of 8.1 kPa (60 mmHg, 0.08 bar) was achieved at 4 minutes, insufficient time for the glucose to change. These samples underwent identical handling and measurements. This method resulted in two plasma glucose measurements of the same blood sample at PO_2 159 kPa (1,200 mmHg, 1.6 bar) and 8.1 kPa (60 mmHg, 0.08 bar) on three separate instruments.

To test within-run precision, the same blood sample was measured 10 times by each glucose instrument. This was performed on three samples having different glucose concentrations: low, normal and high. The YSI measurements at PO_2 8.1 kPa (60 mmHg, 0.08 bar) were used as the standard for bias and imprecision. Bias is defined as the mean of the difference between simultaneous measurements by two methods.[10] Imprecision is defined as the standard deviation of these differences.[11]

Results

PRECISION

Within-run precision was determined by measuring glucose ten times on each of 3 samples containing lithium heparin by each instrument. The results are shown in Table 1.

METHOD COMPARISONS

YSI

The YSI glucose measured in blood at PO_2 159 kPa (1,200 mmHg, 1.6 bar) compared to measurements at PO_2 8.1 kPa (60 mmHg, 0.08 bar) were higher by 1.8 ± 1.6 mg/dl [0.10 ± 0.09 mmol/l (bias ± imprecision)] see Figure 2.

TABLE 1

WITHIN-RUN PRECISION
MEASURED GLUCOSE CONCENTRATIONS
(mg/dl)

Sample	SSP	YSI
A	41 ± 1	37 ± 1
B	184 ± 3	187 ± 1
C	451 ± 16	445 ± 2

The same blood sample (LiHep tubes) measured 10 times (mean ± SD) by SS and YSI, at 3 different glucose concentrations.

SureStepPro

The SSP, operated at 203 kPa (2 bar) with a PO_2 of 8.1 kPa (60 mmHg, 0.08 bar) overestimated glucose by 6.8 ± 5.7 mg/dl [0.38 ± 0.32 mmol/l (bias ± imprecision)] compared with the YSI. At PO_2 159 kPa (1,200 mmHg, 1.6 bar), the SSP underestimated blood glucose by 0.4 ± 7.1 mg/dl [0.02 ± 0.39 mmol/l (bias ± imprecision)] (Figure 2). Figure 3 shows glucose concentrations measured in blood by the SSP operated at 203 kPa (2 bar), a PO_2 of 159 kPa (1,200 mmHg, 1.6 bar) were lower by 3.5 ± 4.5 mg/dl [0.19 ± 0.25 mmol/l (bias ± imprecision)] compared with SSP values at 1 bar. Figure 4 shows the overall bias and imprecision for each condition described relative to the YSI glucose measured at PO_2 8.1 kPa (60 mmHg, 0.08 bar).

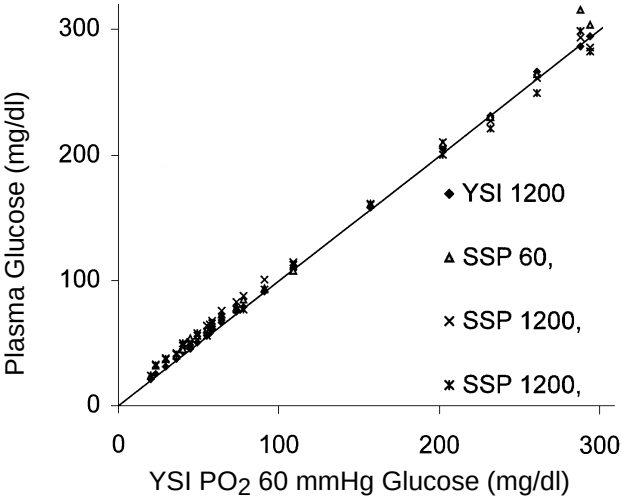

Figure 2. The effect of PO_2 on plasma glucose measurements. The results of all PG measurements of the same blood sample under each condition described: YSI at PO_2 at 159 kPa (1,200 mmHg, 1.6 bar), SS at PO 8.1 kPa (60 mmHg, 0.08 bar) and at159 kPa (1,200 mmHg, 1.6 bar) at 101 kPa (1 bar), and the SS at PO_2 159 kPa (1,200 mmHg, 1.6 bar) at 203 kPa (2 bar) against YSI at PO_2 at 8.1 kPa (60 mmHg, 0.08 bar) (line of idenity shown). The numbers against YSI and SSP are the pressures in mmHg of PO_2.

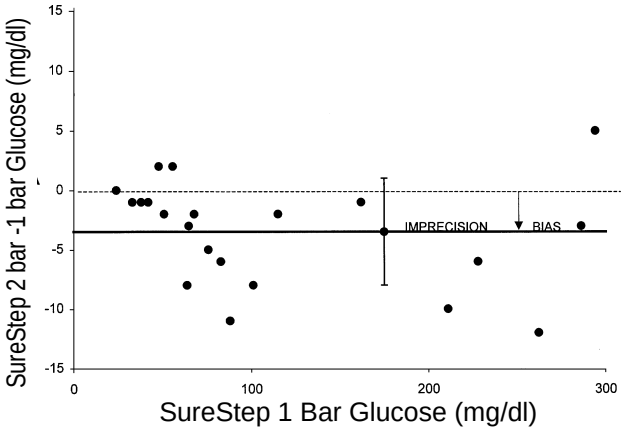

Figure 3. SS performance at 203 kPa (2 bar) compared with 101 kPa (1 bar) with PO_2 159 kPa (1.6 bar, 1,200 mmHg). Plasma glucose values were lower by 3.5 ± 4.5 mg/dl (bias ± imprecision).

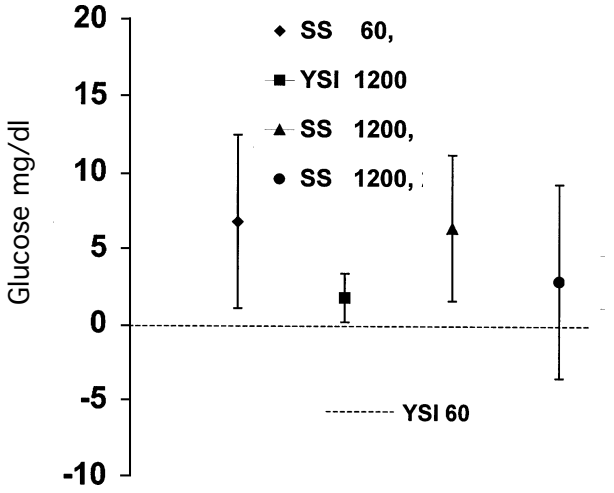

Figure 4. Displays Bias and Imprecision for each conditon described relative to the gold standard of YSI PG measurements at PO_2 8.1 kPa (60 mmHg, 0.08 bar). From the left the bars are SS at PO_2 8.1 kPa (60 mmHg, 0.08 bar), then YSI and SS at PO_2 159 kPa (1.6 bar, 1,200 mmHg) at 101 kPa (1 bar) and on the right SS at PO_2 159 kPa (1.6 bar, 1,200 mmHg) at 203 kPa (2 bar). The numbers against YSI and SSP in the graph above are the pressures in mmHg of PO_2.

EFFECT OF HEPARIN AND FLUORIDE

In similar samples containing either sodium fluoride or lithium heparin, plasma glucose measured by the YSI showed a mean difference (± SD) of only –0.6 ± 3.8 mg/dl [-0.03 ± 0.21 mmol/l (9 samples)], whereas simultaneous measurements of glucose by the SSP in 11 samples containing sodium fluoride showed a mean difference (± SD) of (56 ± 40 mg/dl (3.1 ± 2.2 mmol/l) versus YSI.

Discussion

This is the first study that has evaluated blood glucose measurements using both a bedside glucometer and a laboratory standard instrument under defined PO_2 conditions. Previous studies using laboratory instruments have either incomplete documentation of their results,[2] or not stated the time delay between glucometer and laboratory reading[4] or not used a laboratory instrument as a reference.[3] The time delay is important because PG decreases in blood over time (Figure 5). Our study has minimised the effects of glycolysis in samples by rapidly tonometering blood from PO_2 159 kPa (1,200 mmHg, 1.6 bar) to 8.1 kPa (60 mmHg, 0.08 bar) in 4 minutes, and measuring glucose within 2 minutes thereafter.

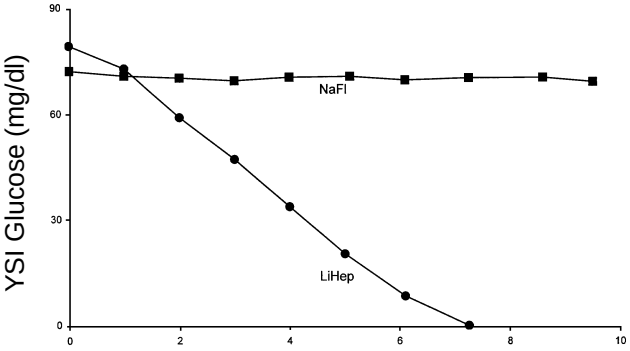

Figure 5. Serial plasma glucose measurements of the same sample by YSI in NaF and LiHep tubes over time.

Glucometers in the past have performed unsatisfactorily under a number of environmental conditions: hyperbaric,[1-3] humidity,[5-7] altitude[7] and low PO_2.[7] Shafer showed a significant difference between reaction rate and reaction end point glucometers; with the reaction rate technique performing better.[2] Her conclusion was, that since the reaction rate method made an estimation (at 20-30 seconds) of a glucose end point, this allowed a shorter strip exposure time to the high PO_2. The SSP chemistry is based on a reaction endpoint but was designed to allow the reaction site to be exposed to ambient oxygen.

The SSP enzymatic reactions occur at the bottom of the test strip that is open to ambient atmospheric oxygen and opposite to the site where blood is applied.[11] This design allows oxygen to exist in excess for the reaction. With the availability of ambient oxygen, the blood sample PO_2 is not reaction rate limiting. SSP monitors the reaction for its endpoint with glucose as the only rate-limiting substrate.

Other designs of test strip formats may make access for oxygen difficult. For example, Accuchek Easy (Boehringer Mannheim Inc, USA) is a system in which the reagent is coated onto a plastic window and sample is supplied from above. The colour formed is viewed from below through the window. In these systems oxygen must get to the reaction site primarily by diffusion through the sample because it can not easily pass through the window.[12] To obviate this, the manufacturers utilise a dye system that essentially takes the place of oxygen in the reaction.[13] Doing so provides for a very fast colour development but may create an oxygen dependency because the oxygen content in the sample may compete with glucose in the oxidation reaction.

Some other glucose meter systems based on glucose oxidase incorporate a mediator molecule or compound in the strip chemistry. This mediator shuttles electrons from the oxidation of glucose to the electrodes. Because oxygen can also perform this function, there can be competition between oxygen and the mediator for electrons. The test strips are factory calibrated with a certain level of blood PO_2 and this level must be relatively consistent with the oxygen level of blood samples. If blood samples have relatively lower or higher PO_2 levels, test inaccuracies could result.

For the YSI, glucose in the sample is stirred and diluted in the sample chamber. Glucose then diffuses through a thin polycarbonate membrane. Once past the polycarbonate membrane, glucose encounters an extremely thin layer of glucose oxidase. There the following reaction occurs:

Glucose + O_2—-> H_2O_2 + By-product.

Although oxygen is consumed in this reaction, the buffer is not seriously depleted of oxygen, nor is the rate of enzyme reaction very sensitive to small changes in oxygen concentration. Therefore, it is not necessary to measure or control the oxygen content in the sample chamber. Hydrogen peroxide diffuses toward the platinum anode in the probe assembly, where it is electrochemically oxidised, creating current that is measured.[8] The rate of the chemical reaction is limited primarily by diffusion. This results in improved linearity, calibration stability and freedom from enzyme inhibition errors.

In summary, the YSI remained accurate over the glucose range 25-300 mg/dl (1.4-16.6 mmol/l) at two PO_2 values of 8.1 kPa (60 mmHg, 0.08 bar) and 159 kPa (1,200 mmHg, 1.6 bar). The SSP remained within 14% of the YSI at PO_2 8.1 kPa (60 mmHg, 0.08 bar) measured values, within the tested glucose range under PO_2 variation from 8.1 kPa (60 mmHg, 0.08 bar) to 159 kPa (1,200 mmHg, 1.6 bar). Thus being well within the designed performance of the SSP, which is to measure blood glucose values to within 20% of laboratory values. The SSP is also accurate when used inside the hyperbaric chamber at 203 kPa (2 bar). Although the YSI measured glucose accurately in tubes containing sodium fluoride or lithium heparin, the same comparison

with the SSP showed a large error in samples containing sodium fluoride.

References

1 Springer R. The importance of glucometer testing of diabetic patients pre and post dive. *Undersea Biomed Res* 1991; 18 (Suppl): 20

2 Shafer MR. The effect of increased atmospheric pressure on glucose reagent strip accuracy. *Mil Med* 1992; 157 (4): 162-165

3 Price ME Jr, Hammett-Stabler C, Kemper GB, Davis MG and Piepmeier EH. Evaluation of glucose monitoring devices in the hyperbaric chamber. *Mil Med* 1995; 160: 143-146

4 Edge CJ, Grieve AP, Gibbons N, O'Sullivan F and Bryson P. Effects of pressure on whole blood glucose measurements using Bayer Glucometer 4 blood glucose meter. *Undersea Hyperb Med* 1996; 23 (4): 221-224

5 Giordano BP, Thrash W and Hollenbaugh L. Performance of seven blood glucose-testing systems at high altitude. *Diabetes Educator* 1989; 15: 444-448

6 Gregory M, Ryan F, Barnett JC et al. Altitude and relative reagent strips. *Clin Chem* 1988; 34 : 1312

7 Barnett C, Ryan F and Ballonoff L. Effect of altitude on the self monitoring of blood glucose (SMBG). *Diabetes* 1987; 36 (Suppl): 117A

8 Gleason B. *YSI Model 2700 Select Biochemistry Analyser: Users' Manual*. Yellow Springs: YSI Incorporated, 1992.

9 Weaver LK and Howe S. Normobaric measurement of arterial oxygen tension in subjects exposed to hyperbaric oxygen. *Chest* 1992; 102 (4):1175-1181

10 Altman DG and Bland JM. Measurement in medicine: The analysis of method comparison studies. *Statistician* 1983; 32: 307-317

11 Barker SJ. Blood volume measurement. *Anesthesiology* 1998; 89: 1310-1312

12 Ervin K and Kiser E. Issues and implications in the selection of blood glucose monitoring technologies. *Diabetes Technology & Therapeutics* 1999; 1: 3-11.

13 Hoenes J. Colorimetric assay by enzymatic oxidation in the presence of an atomatic nitroso or oxime compound. *US patent 5,334,508*. 1994

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HYPERBARIC OXYGEN THERAPY FOR RADIATION-INDUCED HAEMORRHAGIC CYSTITIS

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Key words

Hyperbaric oxygen, irradiation, treatment.

Summary

A retrospective study of the ten year experience of the Fremantle Hospital Hyperbaric Unit in the treatment of radiation-induced haemorrhagic cystitis. This is the largest reported series in Australia and the second largest found in the literature. The objective of this study is to examine the benefit of a course of hyperbaric oxygen therapy in this condition, for which other treatment modalities are often inadequate, temporary and associated with much morbidity. A majority of patients obtained at least symptomatic benefit with minimal discomfort and no major complications. There was a marked decrease in the requirement for blood transfusion. This suggests that hyperbaric oxygen (HBO_2) therapy in radiation-induced haemorrhagic cystitis is both efficacious and well-tolerated, and should be considered for all patients with this condition. Further trials, with more objective outcome measurements, need to be undertaken.

Introduction

Irradiation is a common therapy for a variety of malignant tumours in the pelvic region. Haemorrhagic radiation-induced cystitis and proctitis are side effects that