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Letter to clinical endocrinology

Title:

Evidence of systematic and proportional error in a widely used glucose oxidase analyzer: Impact for clinical research?

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INTRODUCTION

Real time glycemia is a cornerstone for metabolic research, particularly when performing oral glucose tolerance tests (OGTT) or glucose clamps. From 1965 to 2009, the gold standard device for real time plasma glucose assessment was the Beckman glucose analyzer 2 (Beckman Instruments, Fullerton, CA), which technology couples glucose oxidase enzymatic assay with oxygen sensors. Since its discontinuation in 2009, today's researchers are left with few choices that utilize glucose oxidase technology. The first one is the YSI 2300 (Yellow Springs Instruments Corp., Yellow Springs, OH), known to be as accurate as the Beckman(1). The YSI has been used extensively for clinical research studies and is used to validate other glucose monitoring devices(2). The major drawback of the YSI is that it is relatively slow and requires high maintenance. The Analox GM9 (Analox instruments, London), more recent and faster, is increasingly used in clinical research(3) as well as in basic sciences(4) (e.g. 23 papers in *Diabetes* or 21 in *Diabetologia*).

Although a report from the Analox manufacturer shows good linearity in a wide range of glucose concentrations; data assessing its reliability and agreement in clamp and OGTT conditions are scarce. The aim of this study was to assess whether or not the Analox is accurate to serve as a replacement for the YSI during clamp and OGTT studies. Our goal was to analyze the association, reliability and agreement between the two devices, in order to confirm their interchangeability for clinical research.

METHODS

Two hundred ninety three plasma specimens from 13 OGTT and hyperinsulinemic euglycemic clamps from subjects recruited in our ongoing research study were used for this comparison. All subjects signed the IRB approved consent.

Immediately after drawing, 0.4 ml of blood was placed in microtubes containing 30 I.U. of Lithium-Heparin and 1 mg Sodium Fluoride per ml of blood as glucose preservative. Both of these chemicals are known not to interfere with glucose oxidase measurements. Microtubes were spun in a microcentrifuge and plasma was loaded simultaneously on both the YSI and the Analox. These were previously calibrated as specified by the manufacturers. Calibrations were repeated throughout the OGTT or clamps. Manufacturer's standards of various known concentrations were used to assess quality of calibration throughout the tests. All solutions were kept at 4 degrees Celsius as suggested by the manufacturers.

To analyze absolute differences, paired-sample T-tests were performed between YSI and Analox results. Simple linear regression was used to confirm linear relationship and its dispersion was assessed by standard error of estimation (SEE). To assess repeatability, the regression line was compared to the identity line. Concordance correlation coefficient (CCC), which contains both measurements of precision (p, Pearson correlation coefficient) and accuracy (Cb, bias correction factor), was also computed. To confirm agreement a Bland&Altman Plot was done. Reliability was assessed using intraclass coefficient correlation (ICC), technical error of measurement (TEM) and coefficient of reliability (R). The percentage of TEM (%TEM) was considered as a measure of inter-device coefficient of variation. All analyses were performed using PASW for Windows version 20.0 (SPSS Inc., an IBM Company, Chicago, IL) and MedCalc Statistical Software (MedCalc Software, 12.4.0.0, Belgium). For all tests, statistical significance was set at $P<0.05$.

RESULTS

A mean significant difference of 1.05 mmol/L was found between Analox and YSI ($P<0.001$), indicating a systematic error. Pearson's correlation ($r=0.777$;$P<0.001$) and linear regression ($R^2=0.604$;$P<0.001$) are presented in figure 1A. The SEE was 0.83 mmol/L. A

broad dispersion was observed in the euglycemic range (r=0.341, R^2=0.116, both *P*<0.001) with SEE= 0.86 mmol/L. In values higher than 6.1 mmol/L, dispersion was lesser (r= 0.994, R^2=0.987, both *P*<0.001) with SEE=0.19 mmol/L.

Repeatability results indicated weak precision (p=0.777) and accuracy (Cb= 0.712), with a low CCC (0.554). The Bland&Altman plot (Figure 1B) illustrated that the higher the glycemia, the higher the difference. There was a significant proportional bias (Kendall's Tau=-0.403, *P<0.001*).

Reliability was weak with an ICC of 0.865 (*P<0.001*) and high TEM and %TEM (1.89 mmol/L and 31.9%, respectively). The coefficient of reliability was very low (R=0.234).

DISCUSSION

The main finding of this study was that glycemia measured from YSI and Analox were significantly different with both systematic and proportional errors. To our knowledge this is the only study comparing the Analox to the YSI that can be seen as today's gold standard(5). We did not find an acceptable agreement between the YSI and the Analox. Reliability and concordance were modest. In addition, the Analox overestimated 99.7% of the specimens.

Our results are in disagreement with the manufacturer information comparing 123 specimens measured by Analox and Beckman (http://www.analoxusa.com/analoxgm9info.htm). They reported a well-fitted linear equation Y=1.005·X-0.073 (R^2=0.998) which contrasts with the one reported in this study Y=0.947·X+1.334 (R^2=0.604). This discrepancy may be partially explained by the wide range of concentrations used by the manufacturer to validate the GM9 (from 3.00 to 23.98 mmol/L) and the fact that few samples were in the euglycemic range, which may introduce bias(6) and increase type I error. In contrast, we used human specimens in

physiological range covering euglycemia and hyperglycemia, and increased the sample size by a factor of 2.4.

Although this may not be a problem for clinical diagnostic (it can be assumed that clinical diagnosis is done based on clinical chemistry laboratories using automated machines), it could have major implications for clinical studies. In our ongoing studies, we screen volunteers to exclude diabetic (DM) and include in different groups those that are impaired glucose tolerant (IGT) or normal glucose tolerant (NGT). Based on 2 hours OGTT glycemia and ADA criteria, among 54 subjects screened, 33 were NGT (61%), 18 IGT (33%) and 3 DM (6%) with the YSI, and 18 NGT (33%), 29 IGT (54%) and 7 DM (13%) with the Analox. Thus, if using the Analox, we would have excluded more subjects and unfittingly created study groups.

The main limitations of this study is that we did not compare the YSI and the Analox against the Beckman. A recent study(7) compared another available glucose oxidase device against both the Beckman and the YSI. They determined a complete agreement between the YSI and the Beckman in the range observed by our study (below 13 mmol/L). Although we did not directly assess manufacturer's reagents variability, calibrations were controlled by the use of manufacturer's standards of different concentrations. Sensors were controlled by the manufacturers and did not present signs of damage that could explain the differences observed.

In conclusion, we propose to use caution when using the Analox GM9 for clinical research. The measurement bias observed in this study could have consequences encompassing misinformed categorization in different study groups based on glucose tolerance tests, misinterpretation of glucose kinetics and inability to compare among studies.

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DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

FA instigated the project, researched data, interpreted results and wrote the manuscript. NTB collected data and edited the manuscript. EAC interpreted results and drafted the manuscript.

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FIGURE LEGEND

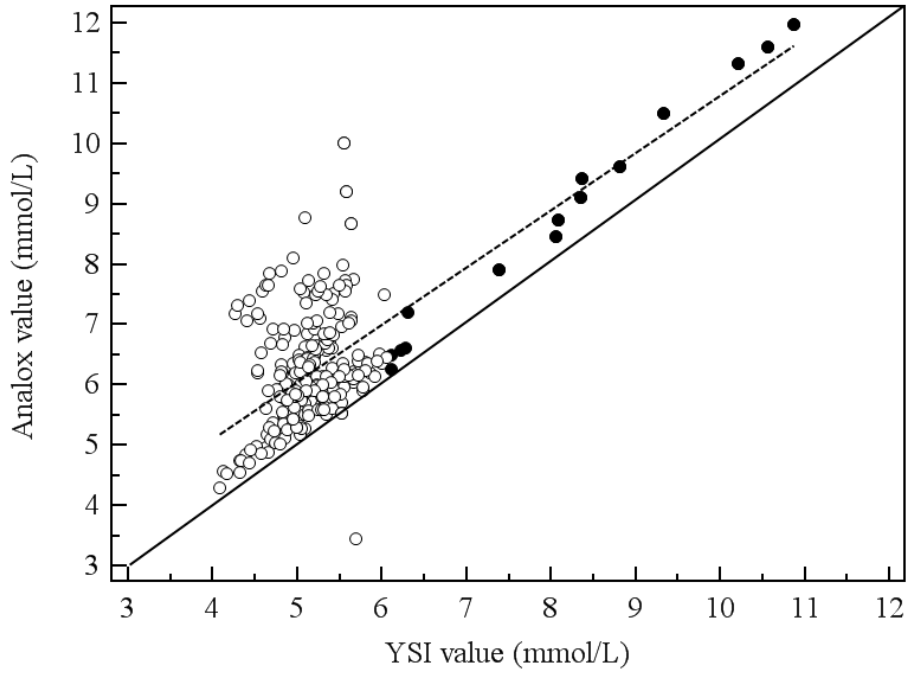
Figure 1.

Panel A Scatter plot of glycemias from Analox and YSI analyzers. White circles=YSI glycemia ≤6.1 mmol/L. Black circles=glycemia >6.1 mmol/L. Dashed line=linear regression line. Solid line=identity line.

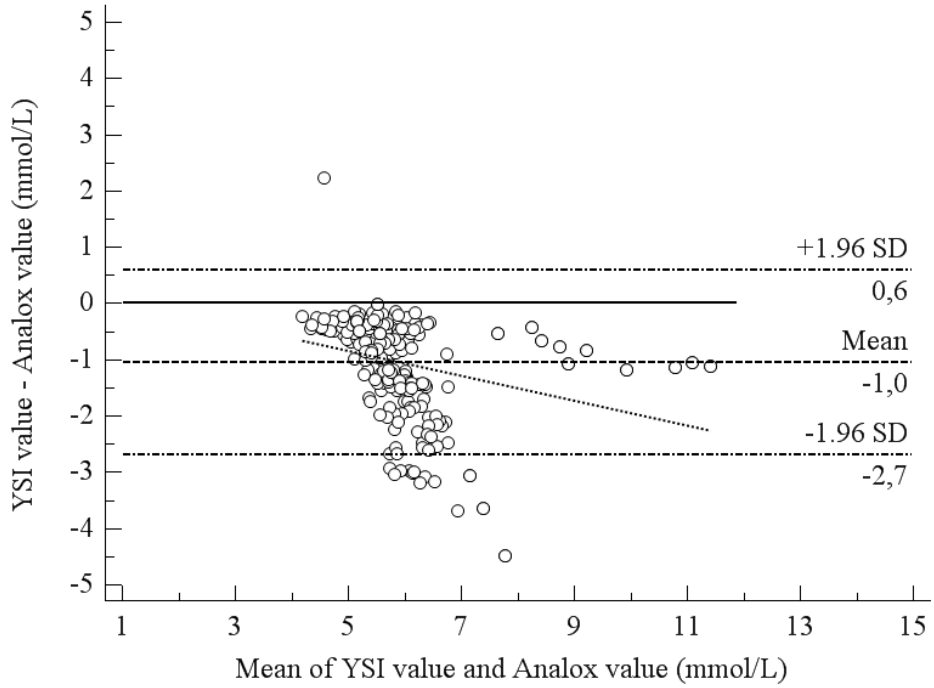
Panel B Bland & Altman plot for agreement analyses between YSI and Analox glucose measurements. Solid line=zero. Dashed line=mean differences between methods (systematic error). Dotted line=trend between differences and means (proportional bias). Dotted-dash lines=intervals of concordance.

Figure 1

A


Analox value (mmol/L)
YSI value (mmol/L)
12
11
10
9
8
7
6
5
4
3
3
4
5
6
7
8
9
10
11
12

B


YSI value - Analox value (mmol/L)
Mean of YSI value and Analox value (mmol/L)
+1.96 SD
0,6
Mean
-1,0
-1.96 SD
-2,7
5
4
3
2
1
0
-1
-2
-3
-4
-5
1
3
5
7
9
11
13
15