Does Methemoglobin from Oxidized Hemoglobin-Based Oxygen Carrier (Hemoglobin Glutamer-200) Interfere with Lactate Measurement (YSI 2700 SELECT™ Biochemistry Analyzer)?

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In this study, we validated the accuracy of lactate measurements (YSI 2700 SELECT™ glucose/lactate analyzer) in the presence of methemoglobin from an oxidized bag of hemoglobin-based oxygen carrier (Met-HBOC), hemoglobin glutamer-200 (Oxyglobin®; Biopure Corp). Different combinations of concentrated L-lactate solution, pooled canine plasma, and Plasmalyte A™ were added to 4 sample groups (1%, 10%, 20%, and 40% Met-HBOC [1.3 g/dL]) to yield linear increases in lactate concentration in consecutive samples. The mean difference between measured and calculated lactate was −5.1 mg/dL (1% Met-HBOC), −5.8 mg/dL (10% Met-HBOC), −4.6 mg (20% Met-HBOC), and −8.5 mg/dL (40% Met-HBOC). The root mean square error was 6.5 mg/dL, 7.4 mg/dL, 6.8 mg/dL, and 10.3 mg/dL, respectively. The Bland-Altman correlation (r) was $r = -0.94$ ($P = 0.01$), $r = -0.91$ ($P < 0.001$), $r = -0.90$ ($P < 0.001$), and $r = -0.94$ ($P < 0.001$), respectively, where $r = 0$ for perfect agreement between measured and calculated values. Results indicate that true lactate levels in the presence of Met-HBOC are underestimated when measured by an YSI 2700 analyzer independent of the amount of Met-HBOC present. When interpreting lactate concentrations from a patient with a HBOC present in plasma, underestimation of true lactate levels may occur unrelated to methemoglobin concentrations.

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Hemoglobin-based oxygen carriers (HBOCs) are novel solutions of cell-free hemoglobin that effectively transport and deliver oxygen to the periphery, thereby serving as alternatives to allogenic red blood cells for use in blood transfusions (1,2). HBOCs have been targeted for use in cases of severe blood loss and hemorrhagic shock secondary to traumatic injury or to treat perioperative anemia during elective surgery. Other investigators have studied interference effects from HBOCs on routine chemistries, therapeutic drugs, coagulation, hematology, and blood bank analysis, but none have looked specifically at oxidized HBOCs in these settings (3–5).

Because cell-free hemoglobin is an avid oxygen scavenger in the absence of reducing enzymes contained within red blood cells, HBOCs exposed to air become rapidly oxidized, increasing the amount of methemoglobin compared with oxyhemoglobin (6). This study validated the accuracy of lactate measurements using a YSI 2700 SELECT™ Biochemistry Analyzer (YSI Inc, Yellow Springs, OH) in the presence of methemoglobin from oxidized HBOC (Met-HBOC) and hemoglobin glutamer-200 (Oxyglobin®; Biopure Corp, Cambridge, MA). The correlation between analyzer-measured lactate and calculated or actual lactate concentration was studied (reference range for lactate [venous] is 4.5–20 mg/dL). We hypothesized that the presence of Met-HBOC would interfere with the accuracy of measured lactate values because Met-HBOC undergoes a rapid redox reaction with hydrogen peroxide that the YSI 2700 uses, in part, to measure lactate. With less hydrogen peroxide reaching the electrode in the analyzer, a smaller

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Table 1. Protocol for Lactate Samples

Sample	Added L-lactate from 150 mg/dL stock (μL)	Plasmalyte A™[a] (μL)	HBOC[b] final concentration = 1.3 g/dL (μL)	Canine plasma (μL)	Final sample volume (μL)	Final calculated [lactate] (mg/dL)
1	0	600	100	300	1000	15
2	40	560	100	300	1000	21
3	80	520	100	300	1000	27
4	120	480	100	300	1000	33
5	160	440	100	300	1000	39
6	200	400	100	300	1000	45
7	240	360	100	300	1000	51
8	280	320	100	300	1000	57
9	320	280	100	300	1000	63
10	360	240	100	300	1000	69
11	400	200	100	300	1000	75
12	440	160	100	300	1000	81
13	480	120	100	300	1000	87
14	520	80	100	300	1000	93
15	560	40	100	300	1000	99

Each sample contained varying amounts of concentrated L-lactate solution, Plasmalyte A™, either 1%, 10%, 20%, or 40% methemoglobin-hemoglobin glutamer-200, and canine plasma. The amount of solution added to each sample is shown. Final calculated lactate values are given.

[a] A balanced salt solution.

[b] Hemoglobin-based oxygen carrier (HBOC), hemoglobin glutamer-200 [bovine] was made to have either 1%, 10%, 20%, or 40% methemoglobin for all samples.

current is generated, and underestimation of actual lactate concentrations could occur.

Methods

We used remainder canine plasma from the University of California at Davis, where Animal Use and Care Administrative Advisory Committee approval was obtained. Different combinations of 80% Met-HBOC and 1% Met-HBOC were mixed to yield 4 groups containing 1%, 10%, 20%, and 40% Met-HBOC. Different combinations of concentrated L-lactate solution (Sigma, St Louis, MO), pooled canine plasma, and Plasmalyte A™ (Baxter, Deerfield, IL), a balanced salt solution (Plasmalyte A™ does not contain any lactate), were added to the Met-HBOC groups to make a linear and constant increase in lactate concentration in consecutive samples (15 mg/dL to 99 mg/dL; 15 total samples per group except for the 1% Met-HBOC group, which had 8 samples because of a shortage of canine plasma). The final HBOC concentration in each sample was 1.3 g/dL (Table 1).

A previously opened bag and a new sealed bag of hemoglobin glutamer-200 were determined to be 80% and 1% methemoglobin, respectively, by co-oximetry (Radiometer ABL 700 Series, Copenhagen, Denmark) on the experiment day. The 80% Met-HBOC had a baseline lactate concentration of 97.8 mg/dL, and the 1% Met-HBOC contained 96.5 mg/dL. Baseline lactate present in canine plasma was 16.6 mg/dL. Duplicate lactate measurements were taken by the YSI 2700, and the average was used for statistical analysis. The data were analyzed using root mean square error of the differences (RMSE; RMSE = $\sqrt{[(\text{mean of calculated} - \text{measured difference})^2 + [\text{SD of the differences})^2]}$) and Bland-Altman statistical analysis to compare the calculated values to the measured values (7,8).

Results

The mean differences between measured minus calculated lactate were −5.1 mg/dL, (9.5% of the overall mean of measured and calculated lactate values) for 1% Met-HBOC, −5.8 mg/dL (10.7% overall mean) for 10% Met-HBOC, −4.6 mg (8.5% overall mean) for 20% Met-HBOC, and −8.5 mg/dL (16.1% overall mean) for 40% Met-HBOC (Fig. 1). The RMSE was 6.5 mg/dL (12% of overall mean), 7.4 mg/dL (13.7% overall mean), 6.8 mg/dL (12.6% overall mean), and 10.3 mg/dL (19.5% overall mean), respectively. The Bland-Altman correlation (r) between measured minus calculated difference versus the average of the measured and calculated lactate was $r = -0.94$ ($P = 0.01$), $r = -0.91$ ($P < 0.001$), $r = -0.90$ ($P < 0.001$), and $r = -0.94$ ($P < 0.001$), respectively, where Bland-Altman $r = 0$ for perfect agreement between measured and calculated values.

Discussion

Calculated lactate was the term we used for what the true lactate level should be, taking into consideration everything containing lactate that was added to the

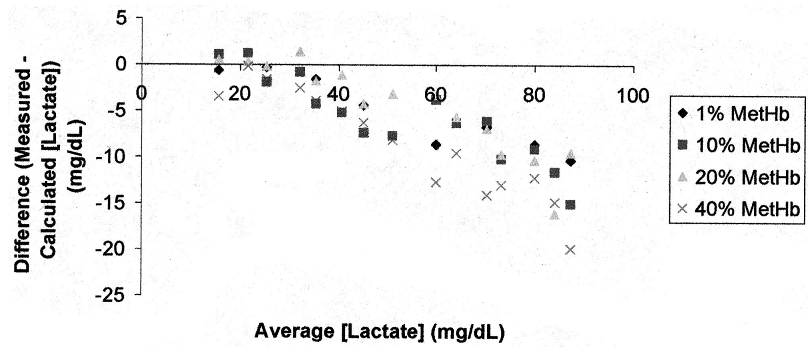

Figure 1. Lactate interference by oxidized hemoglobin-based oxygen carriers (HBOCs). Bland-Altman plots for samples containing hemoglobin glutamer-200 (Oxyglobin®), added L-lactate, Plasmalyte A™, and canine plasma tested on a YSI 2700 SELECT™ glucose/lactate analyzer. The difference between analyzer-measured and calculated lactate concentration versus the average of measured and calculated lactate for each different series of methemoglobin (MetHb) concentration (1%, 10%, 20%, and 40%) is plotted. The reference range for lactate (venous) is 4.5–20 mg/dL.

test samples. This included the baseline lactate levels contained in the initial preparation of hemoglobin glutamer-200, the lactate present in canine plasma, and different amounts of lactate added from a concentrated stock solution. Measured lactate was the value given by the YSI 2700 after analyzing a given test sample.

In comparing measured to calculated values for every test sample for each concentration of Met-HBOC, results indicate that actual lactate levels in the presence of Met-HBOC were consistently underestimated (mean differences were all negative) independent of the amount of Met-HBOC present. Although the 20% Met-HBOC group had the most accurate measured values (mean difference and Bland-Altman correlation coefficient statistic) and the 40% Met-HBOC group had the most negative mean difference and greatest measurement error (RMSE), neither was significantly different from the 1% and 10% Met-HBOC groups. However, all groups significantly underestimated actual lactate concentrations ($P \leq 0.01$).

All hemoglobins, including modified hemoglobins (i.e., HBOCs) in particular, have a propensity to react with hydrogen peroxide (i.e., oxidation of heme Fe) in an enzymatic fashion (6). Biopure Corp. double packs hemoglobin glutamer-200 in airtight containers to prevent oxidation. Usual methemoglobin concentration in an unopened bag is approximately 1%. However, some clinical trials and studies using a similar product have detected increased methemoglobin levels in new products (4).

In the YSI analyzers, lactate is bound by a substrate-specific enzyme, lactate oxidase, which oxidizes lactate, generating an amount of hydrogen peroxide that is directly proportional to the amount of lactate present in the sample. It seems that the HBOCs likely scavenged enough hydrogen peroxide to cause the machine to report a smaller lactate concentration than it should have. Diaspirin cross-linked hemoglobin has been shown to interfere with L-lactate measurement using the Dade ACA IV analyzer (Dade Behring Inc, Wilmington, DE) (5).

Therefore, when interpreting lactate concentrations from a patient with a HBOC present in plasma, underestimation of true lactate levels may occur unrelated to methemoglobin concentrations but as a result of the redox reaction between HBOC and hydrogen peroxide in the lactate analyzer.

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