Ketones, such as β-hydroxybutyrate (β-HB), are important indicators of metabolic condition in animals and have been shown to be linked to several physiological and ecologically relevant factors. They are metabolized from adipose tissue to provide fuel to peripheral tissues, especially those unable to catabolize fatty acids. During fasting, fatty acids are converted to ketone bodies in the liver to spare carbohydrates and proteins from being used as glucose precursors. Ketosis is the physiologic state in which insufficient supply of glucose leads to an increased β-oxidation of fatty acids and the production of ketone bodies (acetone, acetoacetate, and β-HB) as an alternative primary energy source.

In humans, circulating β-HB concentrations correlate better to the degree of ketosis than do other ketone bodies such as acetone and acetoacetate and the American Diabetes Association emphasizes that measurement of β-HB concentrations in blood is preferred for the diagnosis of diabetic ketoacidosis. In ewes and dairy cows, an elevated β-HB concentration is observed at the end of pregnancy and the start of lactation, which are the periods of highest metabolic demand and the highest risk of pregnancy toxemia and the occurrence of ketosis.

β-HB concentration is elevated in the bloodstream due to mobilization of the body’s fat reserves, especially when there is a negative energy balance in lean ewes. β-HB concentrations have also assisted in evaluating nutritional stress prior to slaughter in livestock.
β-HB analysis has been used to assess the energetic state and energy intake in birds and is associated with fasting and loss of body mass. β-HB concentrations are important indicators of metabolic condition and have been shown to be linked to several ecologically relevant factors including migratory decision-making, starvation, and parasitic load. They provide a metric for monitoring a bird’s metabolic rate and can therefore be an indicator of nutritional state as well as provide important information for physiological and ecological studies. For example, food-deprived vesper sparrows (Pooecetes graminus) also had significantly higher β-HB concentrations than did fed sparrows. King penguins (Aptenodytes patagonicus), like other penguin species, will fast for periods of 5 months in the winter losing about two-thirds of their body weight. Plasma concentrations have been found to be considerably elevated by prolonged fasting and molting due to mobilization of lipid stores in king penguins. Changes in β-HB concentrations have also been used to infer impacts of ectoparasite load and to improve options for antemortem diagnosis of certain diseases. For example, penguins with Aspergillus fumigatus infection had significantly elevated lipoproteins, fatty acids, and ketone bodies, including β-HB, compared with healthy birds, using protein nuclear magnetic resonance spectroscopy and multivariate analysis. These elevations of β-HB are believed to indicate behavioral and/or metabolic adjustments directed to spare energy and body protein during infections and may provide reliable evidence for the diagnosis of certain diseases such as aspergillosis in penguins.

Traditional laboratory assays require serum or plasma, which must be sent to a reference laboratory refrigerated, and freezing is required for long-term storage. However, preparing samples under field conditions is cumbersome as it requires centrifugation and maintaining a cold chain during transportation of samples to the laboratory. Early, rapid, and accurate measurement of indicators of fat metabolism of birds in the field is therefore critical to understand the physiologic and behavioral response of environmental changes. Point-of-care (POC) devices provide an alternative to laboratory-based assays and have broad applications for potential use in veterinary medicine. These devices eliminate delays in obtaining results so that clinicians can make clinical decisions in real time without the challenges of transporting samples from remote locations, reducing response time and risks of sample deterioration. In addition, POC devices typically require small volumes of samples, which can be useful for some cases. Furthermore, the costs of POC devices and their test strips are usually a fraction of that of standard analyzers. The retail value of a commercially available single β-HB measurement at a veterinary reference laboratory is comparable to the cost of 30 test strips for use in a POC device. This provides a financial incentive for using POC devices should they reliably yield accurate and precise results.

Therefore, we investigated the agreement between 3 POC devices and a standard reference laboratory for β-HB concentrations in whole blood (WB) and heparinized plasma samples from African penguins (Spheniscus demersus). We also investigated the effect of the animal age and sex, PCV, serum total solids (TS) concentration, and sample β-HB concentrations as well as type of POC device on the agreement between the POC device and reference laboratory. Lastly, we investigated the precision of 3 POC devices for measuring β-HB in African penguin plasma samples.

Materials and Methods

Animals

Forty-eight systemically healthy African penguins under managed care underwent routine annual physical examination and blood collection for hematology and plasma biochemistry. All animals were examined and sampled in March. Only 1 animal had begun to show signs of molt but had not started premolt fast. This project was approved by the Adventure Aquarium Research and Animal Care Committee (protocol No. 2021-002).

Devices

At the reference laboratory, β-HB concentration was measured using a laboratory unit (Vitros 5600 Analyzer; Ortho Clinical Diagnostics). The reagents (β-Hydroxybutyrate LiquiColor Assay; EKF Diagnostics, Stanbio Laboratory) were assessed by quality control testing and the analyzer was maintained per manufacturer instructions. The assay is reportedly linear to 4.5 mmol/L, and the coefficient of variance (CV) is < 5%.

Three commercially available POC blood ketone meters were used in this study. Each meter uses a test strip of the same brand that comes individually wrapped. Each meter determines the concentration of β-HB in a sample electrochemically by detecting an electrical current generated by the reaction of β-HB in a sample with β-HB dehydrogenase contained in the strip. Only the ketone test strips recommended by the manufacturers were used on each device.

Meter A (Kiss My Keto Blood Ketone Monitoring System; Kiss My Keto LLC) requires 0.5 μL of WB, measures β-HB concentration within a range of 0 to 8.0 mmol/L in capillary WB samples with 30% to 60% PCV, and yields results in < 10 seconds. It is not intended for use with plasma, serum, or venous WB, although venous WB samples were used to evaluate the precision of the device according to the package insert.

Meter B (Precision Xtra Blood Glucose & Ketone Monitoring System; Abbot Diabetes Care Inc) measures β-HB concentration within a range of 0.01 to 8.0 mmol/L, requires 1.5 μL of WB, and yields results in 10 seconds. It is intended for use with capillary or venous WB with 30% to 60% PCV.

Meter C (STAT-Site WB Dual Analyte Measurement System; EKF Diagnostics) measures β-HB concentration and requires 1 μL of WB, yielding results in 10 seconds. According to the manufacturer, it can measure β-HB concentration within a range of 0.1 to 8.0 mmol/L in both capillary blood samples.
and heparinized venous blood samples with 10% to 70% PCV.

Accuracy
Each penguin was examined in the morning prior to the first feed. Blood was collected from the right jugular vein using a 22-gauge, 1-inch needle and 3-mL syringe under manual restraint. Each penguin was sampled only once. Blood was transferred immediately to lithium heparin microtainers, and the remainder of blood in the syringe tested on each of the 3 POC ketone meters and results recorded. Within 1 hour of collection, Hct tubes were filled to determine the PCV and TS concentration by refractometry from each sample and the microtainers centrifuged. Two aliquots of each heparinized plasma sample were transferred into cryovials. One aliquot of each sample was shipped overnight on ice to the reference laboratory for determination of β-HB concentration. The other aliquot was frozen at −18 °C for future analysis.

Precision
To investigate the precision of each POC device, 16 heparinized samples were chosen, each from a different penguin, and included those with the 4 highest and the 4 lowest β-HB concentrations as determined by the reference laboratory. Eight additional samples were chosen using a random number generator (www.calculatorsoup.com). Each sample was thawed and analyzed on each of the 3 POC devices, 3 times in immediate succession. At time of analysis, samples had been frozen for 75 to 84 days.

Statistical analyses
Summary statistics were compiled for the measured variables. The agreement analysis was performed using Bland-Altman bias plots and Deming regression. The mean difference (bias), 95% CI of the mean difference, and limits of agreement (LoA) were calculated. The LoA were calculated as ± 1.96 SD centered on the mean difference.26 A systematic bias was considered significant if the 95% CI of the mean difference did not include the value 0.

The association between the difference and the analyte concentration was examined by regressing the difference between the 2 methods and the reference method. A change in bias related to analyte concentration (proportional bias) is shown by the significant slope of such a regression line.26,27 Regression analysis between the 2 methods was performed using weighted Deming regression and the jackknife method for calculation of the CI.

To explore the effect of multiple variables on the difference between the methods, a generalized mixed linear model (GMLM) was built in which the absolute difference was the dependent variable and sex (male or female), age, serum TS concentration, PCV, β-HB concentrations (reference method), matrix (plasma or WB), and instrument (meter A, B, or C) were included as fixed effects, while accounting for individual penguin as a random effect.

Imprecision (random error) from the 3 POC meters was measured calculating the intra-assay CV.28 Total error observed (TEobs) was calculated as indicated by the American Society for Veterinary Clinical Pathology: total error observed = 2 CV + bias (%).

Data analyses were performed and figures created using statistical software programs (SPSS Statistics, version 24; IBM Corp; and R 3.6.3, R Core Team, 2020; www.R-project.org). Two tailed P values < 0.05 were considered significant.

Results
One sample was obtained from each of 48 African penguins during the study period. Of these, 46 included PCV and serum TS concentration and comprised the population included in the GMLM. The mean PCV was 48% with ranges from 41 to 58%. The mean serum TS concentration was 6 g/dL with ranges from 5 to 7 g/dL. Of the 48 African penguins included, 26 (54%) were females and 22 (46%) were males with ages ranging from 3.6 months to 33.5 years and a median age of 4.6 years.

Agreement
The relationships between β-HB measured with the POC devices and the reference laboratory were displayed graphically (Figure 1). β-HB concentration values obtained with the POC and the reference standards, manual PCV, and serum TS concentrations were tabulated (Table 1). Considering the lack of linear relationship between the results from meter C and those from the reference laboratory for plasma samples, the numerical results were not reported.

The differences between the POC devices and the reference standard are displayed graphically as Bland-Altman agreement plots (Figure 2) and tabulated (Table 2). The mean difference for meter A for WB
**Table 1**—Whole blood (WB) and plasma β-hydroxybutyrate concentrations (mmol/L) obtained with a reference laboratory standard analyzer and 3 point-of-care (POC) devices (meters A, B, and C) used to analyze samples from 48 healthy African penguins (*Spheniscus demersus*).

| Device               | Mean ± SD  | Median | Minimum | Maximum |
|----------------------|------------|--------|---------|---------|
| Reference laboratory | 0.64 ± 0.25| 0.62   | 0.33    | 1.72    |
| Meter A WB           | 0.83 ± 0.30| 0.80   | 0.30    | 2.10    |
| Meter A plasma       | 1.87 ± 0.46| 1.70   | 1.20    | 3.50    |
| Meter B WB           | 1.07 ± 0.34| 1.10   | 0.40    | 2.70    |
| Meter B plasma       | 0.91 ± 0.46| 0.80   | 0.30    | 2.70    |
| Meter C WB           | NA         | NA     | NA      | NA      |
| Meter C plasma       | NA         | NA     | NA      | NA      |

NA = Not applicable.

**Figure 2**—Bland-Altman agreement plots for results of analysis of WB (A, C, and E) and plasma (B, D, and F) samples of Figure 1. Circles represent individual measurements. The solid gray line represents the mean difference between the pairs of measurements. The upper and lower dashed gray lines represent the 95% limits of agreement. The solid black horizontal line represents the line of no difference.
Table 2—Results of Bland-Altman regression analysis of β-hydroxybutyrate concentrations (mmol/L) in the samples of Table 1.

| Device   | Mean ± SD difference (mmol/L) | LoA     | 95% CI       | Median | Range        | Correlation coefficient | P value |
|----------|-------------------------------|---------|--------------|--------|--------------|-------------------------|---------|
| Meter A WB | 0.19 ± 0.20                   | -0.21 to 0.59 | 0.13–0.25* | 0.19   | -0.36 to 0.86 | -0.09                   | 0.543   |
| Meter A plasma | 1.23 ± 0.30               | 0.64 to 1.82     | 1.14–1.31* | 1.16   | 0.87 to 2.23  | 0.45*                   | 0.001*  |
| Meter B WB | 0.43 ± 0.14                   | 0.15 to 0.70     | 0.39–0.47* | 0.44   | 0.06 to 0.98  | 0.45*                   | 0.001*  |
| Meter B plasma | 0.27 ± 0.22               | -0.17 to 0.70     | 0.21–0.33* | 0.25   | -0.06 to 0.98 | 0.90*                   | < 0.001*|
| Meter C WB | 0.27 ± 0.14                   | -0.02 to 0.56     | 0.23–0.31* | 0.27   | -0.12 to 0.53 | -0.38*                  | 0.009*  |
| Meter C plasma | 0.82 ± 0.77              | -0.73 to 2.37     | 0.59–1.04* | 0.27   | 0 to 1.91     | 0.48*                   | 0.001*  |

Data are reported as number and percentage of measured, except where indicated.
LoA = Limits of agreement.
*Statistically significant (P < 0.05).

Figure 3—Results of Deming regression analysis for results of analysis of the WB (A, C, and E) and plasma (B, D, and F) of Figure 1. Circles represent individual measurements. The jackknife method was employed to calculate confidence intervals (gray area). The regression line was indicated using the solid black line. The line of no difference is indicated by the dashed gray line.
was 0.19 (95% CI, 0.13 to 0.25; LoA, −0.21 to 0.59) and for plasma was 1.23 (95% CI, 1.14 to 1.31; LoA, 0.64 to 1.82). The mean difference for meter B for WB was 0.43 (95% CI, 0.39 to 0.47; LoA, 0.15 to 0.70) and for plasma was 0.27 (95% CI, 0.21 to 0.33; LoA, −0.17 to 0.70). The 95% CIs of the mean difference of all 3 meters on both matrices did not include “0” and were positive, indicating that these all significantly overestimated β-HB concentration relative to the reference standard. Since the 95% CI did not include 0, all 3 meters on both matrices had systematic bias. The slope of the regression fit of the differences versus the reference standard was significant in all except meter A on WB, indicating a proportional disagreement. The correlation coefficient of the Bland-Altman plots was significant (P < 0.05) for all 3 meters on plasma and meters B and C on WB and therefore had proportional bias. Because the coefficient was positive, bias increased as β-HB concentration increased for all 3 meters on plasma and for meter B on WB. In turn, where the coefficient was negative, bias increased as β-HB concentration decreased for meter C on WB.

Results of the regression analysis (Figure 3) showed both proportional and constant bias since the CI of the intercept did not include the value 0 and the CI of the slope did not include the value 1 for the reference laboratory and meter A. The Deming regression showed that bias was not affected by the age (P = 0.18) or sex (P = 0.16) of the animal nor by the PCV (P = 0.35) or serum TS concentration (P = 0.96) of the sample. The Deming regression also showed that bias was greater for plasma compared with WB (P < 0.001) and that the bias increased with increasing β-HB concentrations (P < 0.001). Meter A overestimated β-HB concentration relative to meter C, whereas meter B underestimated β-HB concentration, relative to meter C.

Precision

On plasma, mean intra-assay CVs for the meters were 1.8% (meter A), 3.9% (meter B), and 6.5% (meter C).

$TE_{obs}$

For WB samples, $TE_{obs}$ was 26.4% for meter A, 59.3% for meter B, and 29.1% for meter C. For plasma samples, $TE_{obs}$ was 189.0% for meter A, 34.3% for meter B, and 115.4% for meter C. For plasma, meter B had a fixed error, followed by meter C followed by meter A. For WB, meter A had less error followed by meter C followed by meter B. Overall, WB samples had less error than plasma for all 3 machines.

Factors affecting disagreement

Based on the GMLM, the absolute difference between meters and reference standard increased by 0.46 mmol/L for each increasing 1 mmol/L of β-HB (coefficient, 0.46; 95% CI, 0.25 to 0.68; P < 0.001), increased by 0.46 mmol/L when using plasma and WB (coefficient, 0.46; 95% CI, 0.36 to 0.56; P < 0.001), increased by 0.18 mmol/L when using meter B (coefficient, 0.18; 95% CI, 0.06 to 0.30; P = 0.004), and decreased by 0.19 mmol/L when using meter B (coefficient, −0.19; 95% CI, −0.31 to −0.07; P = 0.003), as compared with when using meter C. The absolute difference between meters and reference standard was unaffected by sex, age, PCV, and serum TS concentration (Table 3).

Discussion

The interpretation of plasma biochemical profiles can be confounded by the methodologies by which samples are analyzed. POC devices have been used in veterinary and human medicine and offer an easy solution to provide rapid results in comparison to standard laboratory methods. Measurement of plasma metabolites like β-HB may be useful in evaluating nutritional and metabolic status and antemortem diagnosis of diseases.

In this study, we observed that β-HB concentration measured with all of the POC analyzers significantly overestimated actual β-HB concentration relative to the reference laboratory. The negative bias as shown by the Bland-Altman plot suggested that β-HB concentrations measured with the POC devices were higher than those measured by the standard reference laboratory for both WB and plasma. The present findings are consistent with those of similar studies comparing β-HB concentration measurements in red junglefowl (Gallus gallus) using a POC ketone meter.29 Although the meter in this study overestimated β-HB concentration levels in comparison to laboratory derived values, random error was low and laboratory versus device values correlated well.30 However, Sommers et al14 validated a POC device used for measurement of β-HB concentrations in grasshopper sparrows (Ammodramus savannarum) and found that it was highly precise and as accurate as the laboratory assay method and therefore ideal

| Model term | Coefficient | 95% CI | P value |
|------------|-------------|--------|---------|
| Intercept  | −0.31       | −1.05 to 0.43 | 0.406 |
| β-HB at reference laboratory (mmol/L) | 0.46 | 0.25 to 0.68 | < 0.001* |
| Plasma WB Referent | 0.46 | 0.36 to 0.56 | < 0.001* |
| Meter A | 0.18 | 0.06 to 0.30 | 0.004* |
| Meter B | −0.19 | −0.31 to −0.07 | 0.003* |
| Meter C Referent | −0.08 | −0.18 to 0.03 | 0.162 |
| Female | −0.08 | −0.18 to 0.03 | 0.162 |
| Male Referent | −0.01 | −0.01 to 0.02 | 0.348 |
| Age (y) | 0.0 | −0.00 to 0.01 | 0.175 |
| PCV (%) | 0.01 | −0.01 to 0.02 | 0.348 |
| TS (g/dL) | 0.0 | −0.12 to 0.12 | 0.963 |

--- = Not applicable.

*Statistically (P < 0.05) significant.
for field conditions. Morales et all also compared circulating β-HB concentrations on 3 different POC devices and found that they all strongly correlated with measurements from the standard laboratory in 18 free-living bird species.

Agreement between the POC devices and the reference laboratory was not affected by sex. The present findings are consistent with those of similar studies, comparing β-HB concentrations between sexes in lesser scaup (Aythya affinis). Likewise, research with Western sandpipers (Calidris mauri) showed that concentrations of β-HB did not differ by age or sex.

Agreement between the POC devices and the reference laboratory was not affected by age. All of the penguins were either adults or fully fledged juveniles, and none were noted to be fasting (eg, as occurs during molt) during the course of this study.

Agreement between the POC devices and the reference laboratory was not affected by the PCV or serum TS concentration of the sample. The PCV of the penguins all fell within the PCV ranges for each POC device for which it was labelled for use and therefore explains why the agreement may have not been affected by the PCV. In contrast, it was shown that in humans, blood ketone readings can be artificially elevated with low hematocrits. However, the impact of hematocrit was minimized as there was an inbuilt correction factor for hematocrit interference.

Precision of POC performance was only evaluated using plasma samples. Package inserts for each device state they are not intended for use on plasma, which was supported by the greater bias found between the POC devices and reference laboratory for plasma samples compared with WB samples. Future studies should investigate the precision of the POC meters on WB samples as the meters were more accurate using WB than they were using plasma. Therefore, WB samples may be more clinically relevant than plasma samples.

In conclusion, these results suggest that WB, not plasma, should be used for measurement of β-HB concentration on these POC meters. These results also demonstrate that meter A is the most precise compared with the other POC devices. These findings suggest the use of POC devices for the measurement of β-HB concentration would be acceptable when benchtop analyzers are not available. On the basis of the American Association for Clinical Chemistry, all 3 fulfilled the precision performance criteria (SD < 0.1 mmol/L for β-HB results < 1 mmol/L and CV% < 10% for β-HB results ≥ 1 mmol/L). However, meter A was the most precise of the 3 POC meters, followed by meter B followed by meter C. According to the American Association for Clinical Chemistry, the total allowable error (TEaS) is < 0.3 mmol/L for β-HB concentrations < 1 mmol/L and TEa < 30% for β-HB concentrations ≥ 1 mmol/L in humans. Meter A showed good correlation with the reference laboratory within the TEa for WB and meter B for plasma. Unfortunately, the American Society for Veterinary Clinical Pathology does not have TEa for β-HB concentrations at the time of writing. This study demonstrates that POC devices may be useful to estimate β-HB concentrations for penguins but that clinicians should exercise caution when comparing results obtained from different sample types or by different clinical analyzers. The samples used in this study included a narrow range of β-HB concentrations, which was expected given the study population included only healthy birds. Therefore, the accuracy and precision of the POC meters as determined in this study may not be applicable to samples for which β-HB is particularly high or low, as may occur in birds that are in ill health or fasting. Further investigation of the clinical application and diagnostic value of circulating β-HB concentrations as determined by POC devices in avian patients is needed.

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