Evaluation of a ferret-specific formula for determining body surface area to improve chemotherapeutic dosing

Krista L. Jones DVM, MS
L. Abbigail Granger DVM
Michael T. Kearney MS
Anderson F. da Cunha DVM, MS
Daniel C. Cutler DVM
Meredith E. Shapiro MAP Stat
Thomas N. Tully DVM, MS
Keijiro Shiomitsu BVSc

OBJECTIVE
To use CT-derived measurements to create a ferret-specific formula for body surface area (BSA) to improve chemotherapeutic dosing.

ANIMALS
25 adult ferrets (19 live and 6 cadavers).

PROCEDURES
Live subjects were weighed, and body measurements were obtained by each of 3 observers while ferrets were awake and anesthetized. Computed tomography was performed, and a 3-D surface model was constructed with open-source imaging software, from which BSA was estimated. The CT-derived values were compared with BSA calculated on the basis of the traditional tape method for 6 cadavers. To further validate CT analysis software, 11 geometric shapes were scanned and their CT-derived values compared with those calculated directly via geometric formulas. Agreement between methods of surface area estimation was assessed with linear regression. Ferret-specific formulas for BSA were determined with nonlinear regression models.

RESULTS
Repeatability among the 3 observers was good for all measurements, but some measurements differed significantly between awake and anesthetized ferrets. Excellent agreement was found between measured versus CT-derived surface area of shapes, traditional tape–versus CT-derived BSA of ferret cadavers, and CT-derived BSA of cadavers with and without monitoring equipment. All surface area formulas performed relatively similarly.

CONCLUSIONS AND CLINICAL RELEVANCE
CT-derived BSA measurements of ferrets obtained via open-source imaging software were reliable. On the basis of study results, the recommended formula for BSA in ferrets would be $9.94 \times (\text{body weight})^{2/3}$; however, this represented a relatively minor difference from the feline-derived formula currently used by most practitioners and would result in little practical change in drug doses. (Am J Vet Res 2015;76:142–148)

Neoplasia is extremely common in ferrets, with a lifetime prevalence of up to 100% in a population.1 Lymphoma is the third most common type of neoplasia diagnosed in this mustelid species. In 1 retrospective study, lymphoma comprised 11.9% of all neoplasms. Chemotherapy is recommended for treatment of lymphoma and other neoplasms (eg, squamous cell carcinoma) in ferrets.3–4 As with most species, chemotherapeutic agents are often administered to ferrets on a mg/m$^2$ basis, which requires an estimate of an animal’s BSA for treatment.3 An inappropriate BSA estimation results in a less optimal calculation of the chemotherapeutic dose, which increases the risk of underdosing and reduced treatment efficacy or overdosing and greater possibilities of adverse effects. Consequently, increasing the accuracy of BSA estimation would increase the chances for treatment success and reduce the risk of iatrogenic complications, which can be life-threatening. A ferret-specific BSA formula would aid substantially in the appropriate administration of chemotherapeutic agents for ferrets with neoplastic conditions.

Currently, BSA estimates and dosage recommendations for ferrets typically are based on charts generated for domestic cats, which in turn often are extracted from a formula established for dogs.6,7 The typical formula is in the following form: \(\text{BSA} = K \times \text{(body weight)}^{0.75}\), where \(K\) is a shape constant, the value of which differs among species.7,8 Therefore, current practice of the use of BSA estimators for cats may yield inaccurate estimates for ferrets. Furthermore, formulas that incorporate a measure of length (eg, the Du Bois equation9) typically have lower error rates.10 To establish a BSA formula, BSA must be measured along with other body measurements (typically body weight and length). Traditionally, BSA has been as-

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
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<tr>
<td>AIC</td>
<td>Akaike information criterion</td>
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<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation coefficient</td>
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<tr>
<td>MSE</td>
<td>Mean square error</td>
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sessed by physically measuring the surface area (eg, skinned specimens, traditional tape preparation, and cadaver molds). However, capabilities for volume acquisition with multislice CT provide the opportunity to assess BSA in a noninvasive, nonlethal manner.

There is an increasing need and opportunity for chemotherapeutic treatment of lymphoma and other neoplasms in ferrets. Thus, the primary objective of the study reported here was to use CT to assess BSA in adult ferrets and to optimize 2 ferret-specific BSA formulas (one based on body weight alone and the other incorporating body weight and another body measurement). It was expected that the latter would provide an improved correlation with the data but that either would provide a superior means for determining chemotherapeutic doses, compared with use of the current feline formula typically used for ferrets. An additional objective was to validate CT measurement of BSA in 2 ways: comparison of CT-derived measurement of surface area to values calculated by use of geometric formulas of simple geometric shapes and comparison of CT-derived BSA to the traditional BSA obtained from tape preparation of ferret carcasses. We also intended to determine whether morphometric measurements obtained when a ferret was awake differed significantly from those obtained when the ferret was anesthetized.

Materials and Methods

ANIMALS

Nineteen live adult ferrets, most of which were client-owned animals, were enrolled in the study. The live ferrets comprised 12 males (11 neutered and 1 sexually intact) with body weights that ranged from 786 to 1,850 g (mean, 1,153 g) and 7 females (all spayed) with body weights that ranged from 620 to 1,080 g (mean, 846 g). Body condition score of ferrets ranged from 2.5 to 4 on a 5-point scale. There were no restrictions for enrollment in the study; however, ferrets deemed to have an increased risk for adverse effects during anesthesia on the basis of medical history or results of physical examination were included only when a CT evaluation was medically indicated and the owner was aware of the increased risk. A minimum sample size of 24 was selected on the basis of the estimated number needed for analysis of morphometric data, with \( \alpha = 0.05 \) and power = 80%, when variation is unknown and the square of the desired significant difference is equal to two-thirds the unknown variance. Therefore, 6 cadavers of adult ferrets (2 males and 4 females) were also included in the study to enable us to compare CT results with results for the traditional tape method. The study protocol was reviewed and approved by the Louisiana State University Institutional Animal Care and Use Committee, and informed consent was obtained from all owners.

BODY MEASUREMENTS

Food was withheld from ferrets for 3 to 6 hours prior to anesthesia. Before anesthesia, a general physical examination was performed on each ferret. Then, measurements that included body weight, ventral body length (tip of nose to anus), dorsal body length (tip of nose to base of tail), dorsal body length with tail (tip of nose to tip of tail), head circumference (measured on a line that passed over both ears), head diameter (widest point), and chest circumference (measured at the xiphoid process) were each recorded by 3 observers. All ferrets were held in a vertical position with the body alignment as straight as possible to enable accurate measurements.

Ferrets were then premedicated with midazolam hydrochloride (0.3 mg/kg, IM) and hydromorphone hydrochloride (0.05 mg/kg, IM). Approximately 15 minutes later, anesthesia was induced with 5% isoflurane in O2 administered via face mask; oxygen flow rate was 2 L/min. Once sufficient muscle relaxation and loss of palpebral reflexes were evident, tracheal intubation was performed, and the eyes of the ferrets were lubricated. Anesthesia was maintained by the administration of 1% to 2% isoflurane with the aid of a Bain circuit. Heat support was provided as needed with a water heating pad and convective air warmer to maintain body temperature between 36.5° and 37.5°C. Anesthetized ferrets were monitored with a capnograph. In addition, body temperature, heart rate, ECG, respiratory rate, oxygen saturation measured with pulse oximeter, and end-tidal CO2 concentration were monitored with a multivariable monitor. Body measurements were then obtained again by the same 3 observers.

CT IMAGES AND RECONSTRUCTION

Computed tomography was performed on anesthetized ferrets by use of a 16-slice helical CT scanner. Each ferret was positioned in sternal recumbency. Briefly, 2.5-mm helical transverse images were obtained from the nasal planum to the tip of the tail. Images were acquired at a pitch of 1.375, 120 kVp, and 200 mAs with a matrix size of 512 X 512; a standard algorithm was used for image analysis. In the first 8 ferrets, anesthesia monitoring equipment caused metal streak artifacts; thus, for the remaining ferrets, the ECG leads and pulse oximeter were removed during CT scanning. Isoflurane was discontinued after the CT scan was complete. Ferrets received isotonic fluids administered SC, and effects of the injectable anesthetic agents were reversed by administration of naloxone and, for ferrets that were still quite sedated at 1 hour after cessation of isoflurane administration, flumazenil. Ferrets were placed in an incubator and monitored for at least 1 hour after recovery from anesthesia.

The CT images were reconstructed into 0.625-mm slices and analyzed with an open-source imaging software program. A semiautomated 3-D surface model was created for each ferret by use of threshold values between -500 and 2,000 Hounsfield units to enable us to select densities that would incorporate all tissues within the ferrets, including the skin and dense cortical bone, and create the most visibly uniform fer-
ret model (Figure 1). Air within a ferret (eg, within the lungs and gastrointestinal tract) was manually removed with a tracing tool to create a solid model for generation of the BSA output. For each model, surface area was reported in mm². The CT images obtained for ferrets instrumented with anesthetic equipment were imported into image-viewing software, and the equipment was manually removed on a slice-by-slice basis prior to uploading into the imaging software program for analysis.

To validate the CT-derived calculation for BSA, 11 geometric shapes were scanned with CT by use of the same acquisition parameters used for the ferrets. Surface areas of these shapes were calculated manually by use of standard equations and compared with surface areas generated by use of the imaging software program.

### TAPE METHOD

Body measurements were obtained on each of the 6 ferret cadavers by 3 observers in the same manner as described for the live ferrets. Computed tomography was then performed for each ferret with and without an endotracheal tube and anesthetic monitoring equipment (pulse oximeter and ECG leads) in place to enable us to determine the influence of manual editing and removal of the equipment for the live ferrets. Each ferret cadaver was then completely shaved. The body, including all appendages, was fully covered with medical tape. Scissors were used to remove the tape from the body of each cadaver and cut it into pieces; the pieces were then laid flat on a piece of paper. A 2 X 2-cm square was also placed on each sheet of paper as a standard of known area; these sheets were then scanned with a standard flat scanner. The outline of each piece of tape was selected, and the pixels within the outlined area were counted with commercial software. The tape-derived BSA was calculated by use of the following equation: tape-derived BSA (in cm²) = (4 X [the total number of pixels of the scanned tape area])/(the total number of pixels of the 2 X 2-cm square).

### DATA ANALYSIS

All analyses were performed with statistical software packages. Values of \( P < 0.05 \) were considered significant. To assess interobserver variation for body measurements among the 3 observers, the reliability procedure was performed with an ICC analysis. An ICC > 0.75 was considered to indicate excellent reliability. To assess measurement differences between ferrets when awake and anesthetized, the Shapiro-Wilk test was performed to evaluate normality of the data. A paired \( t \) test (for parametric data) or Wilcoxon rank sum test (for nonparametric data) was then performed to assess differences between the ferrets when awake and anesthetized, with \( K^2 \) calculated to evaluate the goodness of fit. Linear regressions were used to assess agreement among surface areas for shapes (known vs CT-derived) and cadavers (CT-derived with monitoring equipment vs tape method; CT-derived with and without monitoring equipment); subsequent graphs were generated by use of commercial software.

To generate a ferret BSA model, 2 types of BSA formulas were optimized. One formula was based solely on body weight (BSA = \( K X [\text{body weight}]^{2/3} \)), and the other incorporated both body weight and another body measurement (BSA = \( K X [\text{body weight}]^{a} X [\text{body length}]^{b} \)), where \( a \) and \( b \) are constants (weight and morphometric exponents, respectively) and \( L \) is one of the other body measurements. Parameters were estimated by use of a statistical approach that fitted nonlinear regression models and estimated parameters on the basis of nonlinear least squares or weighted nonlinear least squares; significant differences between awake and anesthetized ferrets for most morphometrics were considered indicative that separate parameter estimates were needed for these states. Values for \( K \) and

<table>
<thead>
<tr>
<th>Body measurement</th>
<th>Awake</th>
<th>Anesthetized</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head circumference</td>
<td>0.943</td>
<td>0.934</td>
<td>0.661</td>
</tr>
<tr>
<td>Head diameter</td>
<td>0.912</td>
<td>0.927</td>
<td>0.100</td>
</tr>
<tr>
<td>Chest circumference</td>
<td>0.941</td>
<td>0.939</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dorsal body length</td>
<td>0.940</td>
<td>0.947</td>
<td>0.172</td>
</tr>
<tr>
<td>Dorsal body length with tail</td>
<td>0.977</td>
<td>0.867</td>
<td>0.037</td>
</tr>
<tr>
<td>Ventral body length†</td>
<td>0.938</td>
<td>0.872</td>
<td>0.026</td>
</tr>
</tbody>
</table>

An ICC > 0.75 was considered to indicate excellent reliability. Results for the Wilcoxon rank sum test were considered significant at \( P < 0.05 \).

*Represents distance from tip of nose to base of tail. †Represents distance from tip of nose to anus.
MSE were then compared among the generated models as well as with the traditional feline BSA formula; formulas with the lowest MSEs were considered to be the best predictors. To corroborate the use of MSE in the choice of model, AIC values were calculated and F tests conducted to compare the formula generated with ferret body weight to formulas generated with ferret body weight plus another body measurement.

Results

BODY MEASUREMENTS

Excellent reliability was evident for all measurements among the 3 observers for ferrets when they were both awake and anesthetized (Table 1). However, significant differences were observed between awake and anesthetized ferrets for most morphometric measurements (ie, head diameter, chest circumference, dorsal body length with tail, and ventral body length).

CT-DERIVED CALCULATIONS

Linear regression analysis revealed excellent agreement (R² = 0.996; P < 0.001; y = 1.022x – 7.289) between the known and CT-derived surface area of the 11 geometric shapes (Table 2; Figure 2). It also indicated excellent agreement (R² = 1.000; P < 0.001; y = 0.995x – 0.131) between BSA of the cadavers calculated when the cadavers were with and without anesthetic monitoring equipment.

TAPE METHOD

Linear regression analysis revealed good agreement (R² = 0.986; P < 0.001; y = 1.053x + 9.541) between BSA of the cadavers determined by use of the tape method and CT (Figure 2).

BSA FORMULAS

All formulas (traditional feline, ferret body weight alone, and ferret body weight plus another body measurement) appeared to perform similarly with respect to MSE and AIC, with no significant differences detected by use of F tests (Table 3). Negative F values reflected situations in which the residual sum of squares of the more complex model was greater than that for the model for ferret body weight alone, which indicated that fit was not improved. Of those models that incorporated another body measurement, head diameter obtained on awake ferrets was selected for comparison of the 3 model types because it had the

Table 2—Surface area (cm²) of various shapes derived by use of CT versus calculated by use of a geometric formula.

<table>
<thead>
<tr>
<th>Geometric shape</th>
<th>Formula</th>
<th>Derived from CT images</th>
<th>Calculated by use of formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cone</td>
<td>πr² + πrl</td>
<td>282.70</td>
<td>276.26</td>
</tr>
<tr>
<td>Cube</td>
<td>6l²</td>
<td>642.77</td>
<td>624.24</td>
</tr>
<tr>
<td>Cylinder</td>
<td>2πr² + 2πrl</td>
<td>506.78</td>
<td>509.64</td>
</tr>
<tr>
<td>Hexagonal prism</td>
<td>6s (a + l)</td>
<td>463.28</td>
<td>461.76</td>
</tr>
<tr>
<td>Hexagonal pyramid</td>
<td>3as + 3sl</td>
<td>253.56</td>
<td>249.60</td>
</tr>
<tr>
<td>Pentagonal prism</td>
<td>5as + 5sl</td>
<td>446.64</td>
<td>457.50</td>
</tr>
<tr>
<td>Pentagonal pyramid</td>
<td>2.5as + 2.5sl</td>
<td>242.46</td>
<td>243.75</td>
</tr>
<tr>
<td>Rectangular prism</td>
<td>2ls + (h[2l + 2s])</td>
<td>276.57</td>
<td>280.80</td>
</tr>
<tr>
<td>Square pyramid</td>
<td>s² + 2sl</td>
<td>358.02</td>
<td>347.36</td>
</tr>
<tr>
<td>Triangular prism</td>
<td>as + (l[t + b + c])</td>
<td>413.49</td>
<td>414.96</td>
</tr>
<tr>
<td>Triangular pyramid</td>
<td>0.5as + 1.5sl</td>
<td>223.27</td>
<td>231.75</td>
</tr>
</tbody>
</table>

a = Apotheum length. b = Side 2. c = Side 3. h = Height. l = Length. r = Radius. s = Side 1.
Table 3—Results for various optimized formulas for determining BSA (in cm²) in ferrets.

<table>
<thead>
<tr>
<th>Body weight only*</th>
<th>Head circumference</th>
<th>Head diameter</th>
<th>Chest circumference</th>
<th>Dorsal body length</th>
<th>Dorsal body length with tail</th>
<th>Ventral body length</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>10.00</td>
<td>9.94</td>
<td>17.48</td>
<td>14.94</td>
<td>9.55</td>
<td>14.35</td>
</tr>
<tr>
<td>a</td>
<td>NA</td>
<td>NA</td>
<td>0.47</td>
<td>0.45</td>
<td>0.40</td>
<td>0.48</td>
</tr>
<tr>
<td>b</td>
<td>0.30</td>
<td>0.42</td>
<td>0.52</td>
<td>0.26</td>
<td>0.07</td>
<td>0.29</td>
</tr>
<tr>
<td>MSE</td>
<td>5,558.0</td>
<td>4,967.7</td>
<td>5,490.6</td>
<td>5,393.5</td>
<td>4,875.5</td>
<td>5,488.3</td>
</tr>
<tr>
<td>AIC</td>
<td>486.1</td>
<td>—</td>
<td>493.7</td>
<td>492.7</td>
<td>487.0</td>
<td>493.7</td>
</tr>
<tr>
<td>F value ‡</td>
<td>—</td>
<td>—</td>
<td>1.67</td>
<td>1.22</td>
<td>1.33</td>
<td>1.66</td>
</tr>
<tr>
<td>P value ‡</td>
<td>1.00</td>
<td>1.00</td>
<td>0.22</td>
<td>1.00</td>
<td>0.39</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Dorsal body length was from the tip of the nose to the anus. Dorsal body length with tail was from the tip of the nose to the tail. Ventral body length was from the tip of the nose to the base of the nose.

*Calculated by use of the following formula: K X (body weight)²/₃, where K is a shape constant and body weight is in grams. ‡Results from F tests comparing other models to ferret body weight only formula; values were considered significant at P < 0.05.

Discussion

The use of CT for BSA calculations is relatively novel, with this approach used in only 1 other veterinary study to the authors’ knowledge. The present study validated use of CT for BSA calculations through 2 methods: comparing CT-derived values with known surface areas of geometric shapes and CT-estimated BSA for ferret cadavers with values obtained by use of a traditional tape method. Furthermore, it also validated that investigators could account for the presence of anesthetic monitoring equipment in anesthetized patients by comparing values for ferret cadavers scanned with and without such equipment. However, the presence of monitoring equipment that must be manually removed from each image greatly increases the amount of time required for analysis. All of these tests had remarkably high agreement (values of R² > 0.986 to 1.000; all P < 0.001). Although there are several methods (eg, tape method or casts) that can be used for cadavers, CT offers a novel method for measuring BSA in live ferrets in a minimally invasive, nonlethal manner. Future studies could be conducted to further explore the possibility of formulas incorporating morphometric measurements other than body weight that use linear morphometric measurements (eg, body lengths) obtained via CT, although the software used in the present study did not allow measurement of round shapes (eg, head or chest circumference).

In several species, investigators have found that BSA formulas are more accurate when they include body weight and another morphometric measurement. However, results of the present study indicated that the widely used traditional feline formula and 2 formulas reported here (optimized body weight–based formula for ferrets and overall optimal formula [body weight with head diameter measured in awake ferrets]) performed similarly, and any of the model formulas could be used for estimation of ferret BSA. Given the significant difference in head diameter measurements between awake versus anesthetized ferrets, ubiquity of scales versus calipers in clinical practices, ease of use of a weight-based formula, and relatively similar performance, there would appear to be no reason to select a more complex formula in lieu of the ferret-based body weight–only formula (ie, BSA = 9.94 X [body weight]²/₃). From a practical perspective, use of one of the formulas from the present study, versus the traditional feline formula, would typically yield little difference for drug administration. For instance, even in the largest ferrets (approx 2.5 kg), administration of doxorubicin (2 mg/mL) at a dose of 20 mg/m² would yield a dose of 3.66 mg (1.83 mL) by use of the ferret body weight–only formula or 3.68 mg (1.84 mL) by use of the traditional feline formula.
which is a barely measurable difference. Nonetheless, the difference could be relevant for certain drugs used at extremely high mg/m² dosages or drugs administered at low concentrations.

Results of the present study could have been influenced by sample selection. First, a larger sample size might have enabled a higher correlation coefficient or incorporation of a wider range of ferret body types. However, some of the studies on which current formulas for companion animals are based involved the use of <10 subjects, and variation in body shapes among ferrets is expected to be much less than that among various breeds of dogs. Second, the ferrets were all adults with a limited range of body weights (620 to 1,850 g), so BSA estimation might not be accurate for juveniles or ferrets at either extreme of the weight spectrum. Thus, such ferrets might be overdosed or underdosed because of inaccuracy of BSA estimation. However, typical body weight for mature ferrets (600 to 2,000 g) is only slightly beyond the range of body weights for the ferrets in the present study, and body weights for neutered animals most commonly seen at veterinary clinics are relatively uniform (800 to 1,200 g) and well within the range of the ferrets reported here. Third, not all participating ferrets were clinically normal, which could have affected BSA, although it should not have affected BSA measurement. However, given that BSA calculations are determined for ferrets with various stages of neoplasia and, thus, various states of apparent health and body condition, the decision was made to not limit enrollment on the basis of strict requirements for health history or body condition to better reflect the relevant clinic population. Fourth, although there were no restrictions on body condition, all of the ferrets were in moderate condition (body condition score of 2.5 to 4 on a 5-point scale), which would not have impacted the efficacy of CI-derived BSA estimates but could have affected the BSA formulas.

Finally, there is controversy over the use of BSA-based dosing in chemotherapy protocols, although that system remains widely used. Clearly, there is a need for clinical trials to investigate the efficacy and pharmacokinetics of specific chemotherapeutics in ferrets to determine recommended protocols and approaches to dosing, whether on a mg/kg or mg/m² basis. However, such studies are extremely expensive in terms of both time and money, and it can be difficult to find a sufficient number of subjects to enroll. Therefore, BSA-based dosing is likely to continue to be used for chemotherapy of ferrets for some time, so understanding the best method for its use is crucial. This study validated a minimally invasive approach to calculating BSA of individual ferrets and addressed the need for a ferret-specific BSA formula to aid in dosing of chemotherapeutics or other agents with a low therapeutic index.

Acknowledgments

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Footnotes


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