Thromboelastography has revealed hypercoagulability in healthy dogs given prednisone.1,2 In humans, an excess of endogenous and exogenous glucocorticoids increases the risk of thromboembolic disease.2–4 This hypercoagulability may be clinically important in dogs with diseases commonly treated with glucocorticoids such as IMHA, which induces a hypercoagulable state5 and predisposition to thromboembolic complications.6,7 Ultralow doses (ie, 0.5 to 1.0 mg/kg/d, PO) of acetylsalicylic acid are commonly administered to dogs with IMHA because of the drug's antiplatelet activity and putative thromboprophylaxis.8 Indeed, a study8 showed an increase in survival rate when acetylsalicylic

### Objective
To determine the effects of oral prednisone administration with or without ultralow-dose acetylsalicylic acid on coagulation parameters in healthy dogs and to assess intraindividual variation in thromboelastography results.

### Animals
14 healthy research dogs and 10 healthy client-owned dogs.

### Procedures
In a randomized controlled trial, research dogs underwent thromboelastography twice (3 days apart), and intraindividual variation in test results was calculated. Dogs were given prednisone (2 mg/kg/d, PO) plus acetylsalicylic acid (0.5 mg/kg/d, PO) or prednisone (2 mg/kg/d, PO) plus a placebo for 14 days, after which thromboelastography and other tests were repeated. Differences from predadministration (baseline) test results between and within groups were compared. In a separate trial, client-owned dogs also underwent thromboelastography twice 2 days apart to assess intraindividual variation in untreated dogs.

### Results
Intraindividual variation in thromboelastography results for research dogs was ≤10% for maximum amplitude (MA) and α angle. In the research dogs, MA and fibrinogen values significantly increased from baseline, whereas percentage lysis 30 minutes after attainment of the MA as well as antithrombin activity significantly decreased within each group. In the dogs that received prednisone plus a placebo, percentage lysis 60 minutes after attainment of the MA was significantly lower than at baseline. For all parameters for research dogs, there was no difference between groups for change from baseline. Intraindividual variation in findings for client-owned dogs was similar to the variation for research dogs.

### Conclusions and Clinical Relevance
Prednisone administration resulted in hypercoagulability in healthy dogs as indicated by an increase in MA and plasma fibrinogen concentration and a decrease in antithrombin activity. Concurrent ultralow-dose acetylsalicylic acid use had no effect on measured thromboelastography values. The high intraindividual variation in some thromboelastography parameters may preclude routine use of this technique in clinical practice. (Am J Vet Res 2012;73:1569–1576)

**Abbreviations**
- aPTT: Activated partial thromboplastin time
- CV: Coefficient of variation
- IMHA: Immune-mediated hemolytic anemia
- Ly30: Percentage lysis 30 minutes after attainment of the MA
- Ly60: Percentage lysis 60 minutes after attainment of the MA
- MA: Maximum amplitude
- PT: Prothrombin time
- TAT: Thrombin-antithrombin complex
acid was administered to dogs with IMHA in conjunction with prednisone and azathioprine, although the results of that study must be interpreted with caution because of its retrospective nature.

In prior studies involving dogs with IMHA in which hypercoagulability was detected via thromboelastography, results were confounded by treatment of many dogs with glucocorticoids prior to blood sample collection. The effect of Hct, a factor known to affect thromboelastographic findings, was not accounted for in those studies. The effects of glucocorticoid and acetylsalicylic acid administration on coagulation are difficult to separate from those of IMHA. A different study involving healthy dogs found that administration of ultralow-dose acetylsalicylic acid, with or without prednisone, had no effect on thromboelastography results.

Thromboelastography is commonly used to assess coagulation in veterinary medicine and appears to be a more sensitive indicator of hypercoagulability than more traditional hemostatic tests. The method measures the in vitro viscoelastic changes that occur during hemostasis and includes evaluation of plasma clotting factors and cellular components, allowing a global assessment of coagulation. The analyzer used for thromboelastography consists of a pin suspended by a torsion wire that is immersed in a cup of blood, which is then oscillated. Clot formation links the cup and pin, and the torque from this linkage is transmitted to the torsion wire and converted to a digital display. In veterinary medicine, citrated whole blood is typically used, with calcium added to the thromboelastography cup to initiate coagulation. Various other clotting activators can also be added, including kaolin, celite, and recombinant human tissue factor.

Although tissue factor–activated thromboelastography was recently validated in dogs and thromboelastography (with various activators) has been used in multiple clinical studies, intra-individual variation in thromboelastography results has not been thoroughly investigated. One study found no significant difference over 3 consecutive days for the thromboelastography parameters R (reaction time), K (clot formation time), α angle, and MA in healthy dogs when citrated recalculated whole blood was used. However, this study was reported in abstract form, and the full methodology and results were not provided in detail.

The primary objective of the study reported here was to determine the effects of prednisone administration with and without ultralow-dose acetylsalicylic acid on coagulation parameters in healthy dogs. A secondary goal was to determine the intra-individual variation in thromboelastography parameters in healthy dogs when citrated, recalculated whole blood was used. We hypothesized that prednisone administration would lead to hypercoagulability in dogs, addition of acetylsalicylic acid would have no effect, and intra-individual variation in values of thromboelastography parameters would be ≤10%.

**Materials and Methods**

Fourteen healthy research dogs (4 male Beagles and 10 mixed-breed females) with a mean age of 1.9 years (range, 1.25 to 2.5 years) and a mean body weight of 11.0 kg (range, 7.9 to 13.1 kg) were included in the study. The study protocol was approved by the Institutional Animal Care and Use Committee of Virginia Tech.

At the beginning of a 2-week period in which the dogs were acclimated to the research housing, each dog was confirmed to be healthy on the basis of results of physical examination, CBC, serum biochemistry analysis, urinalysis, and microscopic fecal examination. During the first week of that period, all dogs received fenbendazole (50 mg/kg, PO, q 24 h for 3 consecutive days). Each dog was fed a commercial maintenance dog food twice daily, except on sample collection days, at which time food was withheld for a minimum of 12 hours prior to sample collection.

After the 2-week acclimation period, the research dogs were randomly allocated to 2 groups of 7 dogs each and 2 initial (baseline) blood samples were obtained 3 days apart. All phlebotomies were performed by the same individual (ALO), who was unaware of group assignment throughout the study. At each collection point, 2 mL of blood was collected via jugular venipuncture (20-gauge needle) into an evacuated tube (subsequently discarded). At the first collection point only, another 2.7 mL was collected into an evacuated tube containing 3.2% sodium citrate for measurement of PT, aPTT, D-dimer concentration, fibrinogen concentration, antithrombin activity, and TAT concentration.

The tubes containing sodium citrate were carefully inverted 5 times to allow mixing of the blood and anti-coagulant. Tubes for coagulation testing were then centrifuged at 1,418 × g for 10 minutes. Plasma was separated and collected immediately and frozen at −70°C for up to 1 month until testing was performed at the Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University.

After baseline samples were collected, all dogs in 1 group of 7 were given prednisone (median dose, 2.2 mg/kg [range, 2.0 to 2.5 mg/kg], PO, q 24 h) and acetylsalicylic acid (pulverized acetylsalicylic acid tablets with 4,000 C of methylcellulose to achieve appropriate capsule sizes; median dose, 0.53 mg/kg [range, 0.30 to 0.63 mg/kg, PO, q 24 h]). The second group was given prednisone (median dose, 2.3 mg/kg [range, 2.0 to 2.5 mg/kg], PO, q 24 h) and a placebo capsule (containing 4,000 C of methylcellulose) identical in appearance to the acetylsalicylic acid capsules. After 14 days of treatment, blood samples were obtained from all dogs and the following tests were repeated once: CBC, serum biochemical analysis, thromboelastography, and determination of PT, aPTT, D-dimer concentration, fibrinogen concentration, TAT concentration, and antithrombin activity.

**Measurement of coagulation parameters**—Determination of PT, aPTT, and clotible (Clauss) fibrinogen concentration in research dogs were performed with an automated clot detection instrument, commercial reagents, and reaction conditions as previously described. Pooled canine plasma (prepared from 20 healthy adult dogs) was used as the fibrinogen assay standard. The fibrinogen content of the standard (330 mg/dL) was measured via a gravi-
metric method.\textsuperscript{19} Antithrombin activity was measured with a functional assay configured to measure the degree of thrombin inhibition (anti–factor IIa assay) with a commercial chromogenic kit\textsuperscript{a} and the manufacturer's automated analyzer.\textsuperscript{a} Antithrombin activities of the test samples were recorded as a percentage of the pooled canine plasma, which had an assigned value of 100%. A quantitative, immunoturbidimetric method was used to measure D-dimer concentration as described,\textsuperscript{20} with the aid of a commercial kit and the manufacturer's human D-dimer standards.\textsuperscript{b} The TAT concentration was measured with a commercial sandwich ELISA.\textsuperscript{c} The assay is configured with cross-reactive rabbit anti-human antibodies and a human TAT standard.\textsuperscript{31,22} The TAT concentration of test plasma samples was logarithmically transformed for reporting purposes.\textsuperscript{32}

With the exception of TAT concentration, all coagulation parameters assessed are reportedly stable in canine plasma at −80°C for >1 year.\textsuperscript{33} Stability of TAT concentrations has not been evaluated in dogs but is stable in human plasma for 1 month at −80°C.\textsuperscript{24}

**Thromboelastography**—Baseline thromboelastography was performed by the same operator (ALO) on the whole blood samples obtained twice from research dogs prior to treatment to determine the degree of intraindividual variation in results between sample collection points. Each sample was run in duplicate in a separate channel of the thromboelastography analyzer\textsuperscript{d} each day as described.\textsuperscript{23} Following blood sample collection, citrated blood samples were left to sit at room temperature (approx 22°C) for 30 minutes, then each tube was inverted 5 times. Immediately afterward, thromboelastography was performed. Briefly, 20 µL of CaCl\textsubscript{2} was placed into plain thromboelastography cups prewarmed to 37°C, then 340 µL of each citrated blood sample was added to each cup.

Thromboelastographic analyses were run for 120 minutes, and the parameters R, K, α angle, MA, Ly30, and Ly60 were measured and recorded as the mean of the values from each of the 2 channels of the thromboelastography analyzer that were run simultaneously for each sample.\textsuperscript{23} On a daily basis, level I and II quality control samples were evaluated and an \( \chi^2 \) test was performed in accordance with the manufacturer's recommendations.

**Follow-up thromboelastography**—Because of unexpectedly high intraindividual variation in some thromboelastography parameters, intraindividual variation was also determined in 10 healthy client-owned dogs with a mean age of 3 years (range, 1.5 to 4 years) and a mean body weight of 23 kg (range, 9.5 to 65 kg). The dogs were determined to be healthy on the basis of results of physical examination, CBC, and serum biochemical analysis; no history of major health problems in the past 3 months; no history of any bleeding or hematologic disorder at any time; no receipt of anesthesia in the 2 weeks prior to the study; and no health problems in the month following the study. The only medications allowed were flea or tick and heartworm preventatives. Phlebotomy and thromboelastography were performed twice on each dog 2 days apart, as described for the research dogs. Each sample was run in duplicate in separate channels of the thromboelastographic analyzer each day. Owner consent was obtained prior to sample collection.

**Statistical analysis**—Mean ± SD values of coagulation and thromboelastography variables were determined. Intraindividual variations within dogs for R, K, α angle, MA, Ly30, and Ly60 were calculated on the basis of data from the 2 baseline blood samples from research dogs and both samples collected from untreated client-owned dogs. This variation was summarized as mean and 95% CI for each variable. Intraindividual variation ≤10% was considered acceptable.

For normally distributed data regarding research dogs, the change in mean baseline values were compared with values after treatment via the paired \( t \) test to evaluate differences within groups and the 2-sample \( t \) test to compare differences between groups. For data that were skewed, posttreatment measurements were compared with mean baseline measurements within each group by use of the Wilcoxon 1-sample test. A Wilcoxon 2-sample test was applied to compare the change from baseline between groups. A value of \( P < 0.05 \) was considered significant. All analyses were performed with commercial software.\textsuperscript{31}

**Results**

**Changes from baseline values in research dogs**—The MA and fibrinogen concentration increased significantly and Ly30 and antithrombin activity decreased significantly from baseline values after treatment of the research dogs concluded, regardless of whether acetylsalicylic acid was administered with prednisone (Table 1). In posttreatment blood samples from dogs that received prednisone alone, the Ly60 significantly decreased from baseline. All other within-treatment group changes from baseline were not significant, nor was there any difference between treatment groups in change from baseline for any parameter evaluated.

The mean intraindividual variation in all research dogs before treatment and in client-owned dogs was ≤10% for α angle and MA and >10% for K, R, Ly30, and Ly60 (Tables 2 and 3). Day-to-day variation in the results for the quality control samples over the entire study period were as follows for the level I quality control samples: R, 11.8%; K, 0%; α angle, 0.4%; and MA, 2.8%. Respective values for the level II control samples were 5.4%, 16.4%, 1.9%, and 5.3%. Both sets of values were similar to those listed in the thromboelastography manual precision calculations (level I: 7%, 0%, 0%, and 3%, respectively; level II: 10%, 14%, 7%, and 5%, respectively).\textsuperscript{b} All thromboelastography values for all quality control samples were within the manufacturer’s reference limits throughout the study.

**Discussion**

Findings in the present study were similar to those in 2 preliminary studies\textsuperscript{1} in which the effects of prednisone administration on thromboelastography results were evaluated in healthy dogs. In those studies, an increase in MA after prednisone treatment was revealed
through tissue factor–activated thromboelastography and citrated, recalcified thromboelastography. Increases in K and α angle as well as decreases in clot lysis were also found.

The mechanism by which hypercoagulability develops after exogenous glucocorticoid administration in dogs has not been fully evaluated. In dogs with naturally occurring hyperadrenocorticism, increases in

### Table 1—Change from baseline values in coagulation and thromboelastography variables in research dogs following treatment with prednisone (2 mg/kg/d, PO) with or without acetylsalicylic acid (0.5 mg/kg/d, PO) for 14 days within and between treatment groups (7 dogs/group).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment Group</th>
<th>Baseline (Mean ± SD)</th>
<th>Change from Baseline (Mean [95% CI])</th>
<th>P value</th>
<th>Difference between groups (Mean [95%CI])</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (min)</td>
<td>Prednisone alone</td>
<td>7.1 ± 0.9</td>
<td>−1.1 (−3.3 to 1.1)</td>
<td>0.27</td>
<td>−3.0 (−6.2 to 0.1)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>6.0 ± 1.0</td>
<td>1.9 (−0.8 to 4.7)</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K (min)</td>
<td>Prednisone alone</td>
<td>2.8 ± 0.5</td>
<td>−0.5 (−1.3 to 0.3)</td>
<td>0.20</td>
<td>−0.3 (−1.7 to 1.1)</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>2.8 ± 1.0</td>
<td>−0.2 (−1.5 to 1.2)</td>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α angle (°)</td>
<td>Prednisone alone</td>
<td>54.2 ± 4.5</td>
<td>5.5 (−2.7 to 13.7)</td>
<td>0.15</td>
<td>4.6 (−7.8 to 16.9)</td>
<td>0.44</td>
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<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>55.6 ± 7.9</td>
<td>0.9 (−10.3 to 12.1)</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA (mm)</td>
<td>Prednisone alone</td>
<td>51.3 ± 4.4</td>
<td>8.5 (4.5 to 12.4)</td>
<td>0.002</td>
<td>−2.5 (−8.2 to 3.2)</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>51.1 ± 8.3</td>
<td>11.0 (5.9 to 16.0)</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ly30</td>
<td>Prednisone alone</td>
<td>3.2 (0.0 to 11.0)*</td>
<td>−2.8 (−11.0 to 0.0)*</td>
<td>0.031</td>
<td>−1.9 (NA)</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>1.7 (0.1 to 9.3)*</td>
<td>−0.9 (−9.3 to −0.1)*</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ly60</td>
<td>Prednisone alone</td>
<td>10.6 ± 9.4</td>
<td>−9.4 (−17.5 to −1.3)</td>
<td>0.030</td>
<td>−3.9 (−12.6 to 4.9)</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>7.2 ± 5.6</td>
<td>−5.5 (−11.0 to 0.0)</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>Prednisone alone</td>
<td>49.4 ± 4.6</td>
<td>−1.7 (−6.3 to 3.0)</td>
<td>0.42</td>
<td>2.4 (−3.8 to 8.7)</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>49.2 ± 5.9</td>
<td>−4.1 (−9.4 to 1.2)</td>
<td>0.11</td>
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<td></td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>Prednisone alone</td>
<td>12.4 ± 1.1</td>
<td>−0.2 (−0.9 to 0.5)</td>
<td>0.50</td>
<td>−1.5 (−3.2 to 0.2)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>12.4 ± 1.0</td>
<td>1.3 (−0.4 to 3.1)</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (s)</td>
<td>Prednisone alone</td>
<td>13.3 (12.1 to 14.6)*</td>
<td>0.0 (−0.7 to 0.5)*</td>
<td>0.56</td>
<td>0 (NA)</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>13.8 (3.7 to 14.4)*</td>
<td>0.0 (−0.4 to 9.4)*</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>Prednisone alone</td>
<td>249.0 ± 57.1</td>
<td>107.7 (20.2 to 195.2)</td>
<td>0.024</td>
<td>−32.9 (−116.2 to 50.5)</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>342.2 ± 78.7</td>
<td>140.6 (107.2 to 173.9)</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>Prednisone alone</td>
<td>113.7 ± 11.7</td>
<td>−17.7 (−31.8 to −3.6)</td>
<td>0.022</td>
<td>−0.7 (−16.3 to 14.8)</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>115.9 ± 14.4</td>
<td>−17.0 (−27.3 to −6.7)</td>
<td>0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-dimer (ng/mL)</td>
<td>Prednisone alone</td>
<td>143.6 ± 142.5</td>
<td>−71 (−209.2 to 65.5)</td>
<td>0.25</td>
<td>16.4 (−159.0 to 191)</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>162.4 ± 154.4</td>
<td>−88.3 (−229.4 to 52.9)</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (µg/L)</td>
<td>Prednisone alone</td>
<td>7 (3 to 193)*</td>
<td>−1 (−190 to 42)*</td>
<td>1.0</td>
<td>6 (NA)</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Prednisone and ASA</td>
<td>10 (4 to 91)*</td>
<td>−7 (−78 to −1)*</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Indicated values were not normally distributed and are therefore reported as median (range).

ASA = Acetylsalicylic acid. NA = Not applicable.

### Table 2—Intraindividual CVs for thromboelastographic findings in blood samples collected twice, 3 days apart, from 14 healthy untreated research dogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean CV (%)</th>
<th>SD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (min)</td>
<td>28.3</td>
<td>21.3</td>
<td>16.0 to 40.6</td>
</tr>
<tr>
<td>K (min)</td>
<td>20.8</td>
<td>16.9</td>
<td>11.0 to 30.5</td>
</tr>
<tr>
<td>α angle (°)</td>
<td>10.0</td>
<td>7.8</td>
<td>5.5 to 14.5</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>5.1</td>
<td>4.4</td>
<td>2.5 to 7.8</td>
</tr>
<tr>
<td>Ly30</td>
<td>75.3</td>
<td>58.8</td>
<td>41.3 to 109.2</td>
</tr>
<tr>
<td>Ly60</td>
<td>58.5</td>
<td>41.3</td>
<td>34.6 to 82.3</td>
</tr>
</tbody>
</table>

### Table 3—Intraindividual CVs for thromboelastographic findings in blood samples collected twice, 2 days apart, from 10 healthy untreated client-owned dogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean CV (%)</th>
<th>SD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (min)</td>
<td>28.3</td>
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</tr>
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</tr>
<tr>
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</tr>
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<td>Ly60</td>
<td>58.5</td>
<td>41.3</td>
<td>34.6 to 82.3</td>
</tr>
</tbody>
</table>
after disease remission. In the present study, it was hypothesized that glucocorticoid administration may have led to hypercoagulability with glucocorticoid administration. The present study aimed to investigate hypofibrinolysis as a cause of hypercoagulability. 

The importance of the decrease in fibrinolysis, addition of fibrinolytic parameters made it difficult to interpret the high intraindividual variation we detected in values for fibrinogen. Unlike in humans, high plasminogen activator inhibitor 1 activity has not been documented in dogs with naturally occurring hyperadrenocorticism. A canine-specific assay is not commercially available. Another possible explanation for the wide range of TAT concentrations is the study dogs differed in their sensitivities to glucocorticoids. Alternatively, dosage or duration of glucocorticoid treatment may have prevented significant differences from being detected, given that dogs with naturally occurring hyperadrenocorticism have a high TAT concentration.

Failure to detect an effect of ultralow-dose acetylsalicylic acid given may have been too low to have an effect on platelets, a study in which whole blood impedance aggregometry was used found impaired platelet aggregation in healthy dogs receiving 0.5 mg of acetylsalicylic acid/kg/d. Another possible explanation is that the effects of prednisone may have negated the antiplatelet effects of acetylsalicylic acid. Platelet function was not independently measured, which is a limitation of our study.

The primary purpose of the present study was to investigate the effects of prednisone administration with and without ultralow-dose acetylsalicylic acid on coagulation parameters in healthy dogs, but some potentially clinically relevant shortcomings of thromboelastography were revealed. The large intraindividual variation in R, K, Ly30, and Ly60 observed during the treatment trial in the study was further investigated in healthy client-owned dogs. This variation was similar in client-owned dogs and remained > 10% for R, K, Ly30, and Ly60.

Biological (intraindividual or within-subject) variation is possible in coagulation test results in humans, and the degree of variation in PT and partial thromboplastin time is consistently < 10%. Day-to-day intraindividual variation was between 4% and 14% in a study involving healthy humans that underwent kaolin- and tissue factor–activated thromboelastography, with the highest variability detected in R values. Intraindividual variation for several thromboelastography parameters in dogs has been reported previously, with values for R, MA, and α angle between 10% and 20%. However, these results cannot be directly compared with those of our study because thromboelastography was performed on frozen plasma with the use of tissue factor activation and because a different method was used to calculate intraindividual variation. A CV ≤ 10% was chosen to define an acceptable degree of variation in our study on the basis of previously reported variation and the opinion that greater variation could lead to misinterpretation of results.

Given that thromboelastography must be performed on whole blood within a standard period after collection to avoid changes in parameters over time and that use of plasma abrogates the advantages of thromboelastography in evaluating the entire coagulation system, including cellular components, thromboelastography was performed on multiple days. The same operator performed all thromboelastographic tests in an identical manner with the same reagents in,
an attempt to minimize variability in conditions between days. Day-to-day variability in the quality control samples was also calculated, and results were similar to those listed in the thromboelastography manual precision calculations. The variability in results for the quality control samples throughout the study illustrated the inherent variability in the analyzer, reagents, and operator, which explains some of the variability in the actual results.

The similarity in quality control variation between the thromboelastography manual and the present study does suggest that both analyzer and operator error are unlikely to be causes of the marked intra-individual variation in some thromboelastography parameters. To the authors' knowledge, prior evaluation of intra-individual variation in some thromboelastography parameters is limited to an abstract in which no significant differences over a 3-day period were reported within any parameter when citrated recalcified whole blood was used with or without kaolin activation. However, details of the methods and results were not stated. The CVs of duplicate samples (2 channels of the same thromboelastography analyzer) have been evaluated in dogs with various activators (tissue factor and kaolin), but indicators of fibrinolysis were not measured and CVs of duplicate samples are not equivalent to intra-individual variation that was the focus of the present study.

Other factors that can affect thromboelastography results include the interval between blood sample collection and test initiation, operator variability, low Hct, platelet counts, and function, sample hemolysis and dog breed. We attempted to minimize the presence of these factors through careful performance of jugular venipuncture, uniform performance of thromboelastography (same operator at same interval after sample collection), close monitoring of pre- and posttreatment Hct values to ensure they were within reference limits, and intentional inclusion of predominantly mixed-breed dogs. Because of the high incidence of platelet clumping encountered, accurate platelet counts could not be determined for many of the dogs involved in the treatment trial and therefore were not included in the statistical analysis. However, all platelet counts were estimated to be within reference limits at baseline and after treatment.

That the variability in R was high in the present study was not unexpected, considering that this parameter is most influenced by the point at which data collection begins after a blood sample is placed in the thromboelastography cup. In a study of rotational thromboelastometry in which recalcification was used alone and with different activators, coagulation time (equivalent to R) was significantly different between all activators and was most prolonged when recalcification was used as the only activator. In addition, when recalcified whole blood alone was used, results were more affected by sample handling than when recalcified whole blood was used with other activators. Significant differences in results between operators with use of citrated recalcified whole blood and the same thromboelastography analyzer for the parameters R, K, and angle have been documented in a study involving horses, and these differences were minimized when tissue factor activation was used. The use of recalci-

A study of thromboelastography in dogs similar to the rotational thromboelastometry study is imperative to properly interpret the results of clinical studies involving thromboelastography as well as standardize thromboelastographic methods for future studies. The human medical profession is facing similar challenges with both thromboelastography and rotational thromboelastometry, and a working group has been established in an attempt to standardize the methodology and evaluate reproducibility and consistency of related results.

The extreme variation in the fibrinolysis values in the present study was unexpected. Some dogs had markedly high pretreatment Ly30 and Ly60 values, which in some situations was not detected when testing was repeated. A notable lack of reporting of Ly30 and Ly60 values exists in veterinary research, even though thromboelastographic analysis has been performed for a sufficient duration to generate lysis results (90 to 120 minutes) in many studies. Although outlying percentage lysis values could have been excluded from our analysis, we believed it was appropriate to include all values, given the lack of information on fibrinolysis in the veterinary literature. In a comparison of thromboelastography results for Ly60 between Greyhounds and non-Greyhounds, the range of values obtained was large (0% to 19.2% for Greyhounds and 0% to 8.6% for non-Greyhounds). In healthy cats, the mean ± SD Ly60 for citrated recalcified blood is reportedly 7.86 ± 10.21%, and some cats have a marked increase in fibrinolytic values, whereas others have minimal indication of fibrinolysis. It was theorized that the disparity in findings was attributable to platelet retraction. Additional studies are needed to determine whether this markedly increased fibrinolytic pattern is common and whether measurement of thromboelastographic fibrinolytic parameters is reliable in dogs and cats.

In light of the high intra-individual variation observed in the present study, it is important to interpret the changes from baseline values within the groups with caution, particularly with respect to R, K, Ly30, and Ly60. However, the variability in MA was low; therefore, we believe that the hypercoagulability suggested by an increase in MA within the groups is reliable. The small sample size may have been partly responsible for the high SDs and thus high coefficients of variation for some of the parameters, and another study with a larger sample size is needed to validate the findings reported here.


c. STA Compact, Diagnostica Stago Inc, Parsippany, NJ.

d. Dade Actin FS, Dade Behring, Newark, Del.

e. Thromboplastin LI, Helena Diagnostics, Beaumont, Tex.

f. Fibrinogen, Diagnostica Stago Inc, Parsippany, NJ.
References


