The evaluation of conditions associated with thrombophilia (ie, the propensity to develop thrombosis) is of great interest in human medicine and recently also in veterinary medicine.1 In humans, many factors, including exercise, result in thrombophilia.2 A study2 in humans has revealed that exercise results in activation of coagulation and subsequent counter-regulation of the previously initiated coagulation reaction. Exercise-induced activation of the coagulation system is possibly caused by an increased immunologic response attributable to muscular microtrauma,3 which has been observed after strenuous exercise in athletes.4 In vivo examinations have revealed that inflammatory reactions induced by macrophages activated with lipopolysaccharides may activate the coagulation system.5 There was evidence of an increase in coagulation activity in runners by 30 minutes after completing a marathon, and that increase persisted until the next morning.6 Marathon-induced changes in coagulation included a shortening of OSPT and activated partial coagulation time as well as a significant increase in physiologic inhibitors of coagulation (protein S, protein C, and AT) and a significant increase in the plasma concentration of fibrin D-dimer indicative of increased fibrinolysis.6

Maximum exercise such as running a marathon as well as prolonged submaximal physical exercise on a treadmill results in evidence of an increase in fibrinolysis attributable to activation of the coagulation sys-

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**Effect of submaximal aerobic exercise on platelet function, platelet activation, and secondary and tertiary hemostasis in dogs**

Natali B. Bauer, Dr med vet; Elif Er, DVM; Andreas Moritz, Prof Dr med vet

**Objective**—To investigate whether submaximal aerobic exercise in dogs is followed by activation of all phases of coagulation as has been reported for humans.

**Animals**—9 healthy Beagles.

**Procedures**—30 minutes before dogs were exercised, a 16-gauge central venous catheter was placed in a jugular vein of each dog by use of the catheter-through-the-needle technique. Samples were collected before exercise, after running on a treadmill (6 km/h for 13 minutes), and at 60 minutes. Platelet activation was evaluated with platelet morphology indices (mean platelet component, mean platelet volume, and number of large platelets) provided by a laser-based hematology system. Platelet function was assessed in hirudin-anticoagulated whole blood with an impedance-based aggregometer with collagen as the agonist (final concentrations, 0, 1.6, 3.2, 5, and 10 µg/mL). Prothrombin time, activated partial thromboplastin time, and concentrations of fibrinogen, factor VIII, antithrombin, protein C, protein S, and fibrin D-dimer were determined automatically. Kaolin-activated thromboelastography variables R (reaction time), K (clot formation time), angle α, maximal amplitude, and G (clot stability) were measured in recalcified citrated whole blood.

**Results**—Exercise resulted in a significant decrease in mean platelet volume and the number of large platelets but did not change the mean platelet component, which reflected platelet activation as well as platelet function. Secondary and tertiary coagulation did not change significantly, nor did thromboelastography variables.

**Conclusions and Clinical Relevance**—Aerobic exercise resulted in a decrease in the number of large and thus most likely activated platelets but otherwise had no major impact on coagulation in dogs. (Am J Vet Res 2012;73:125–133)
tem. In addition to activation of secondary hemostasis and fibrinolysis, there is evidence of exercise-induced platelet activation in healthy athletes as reflected by unique platelet morphology indices determined by use of a laser-based hematology analyzer. During each platelet activation in healthy athletes as reflected by fibrinolysis, there is evidence of exercise-induced circadian effects on variables.

In human athletes, exercise results in a decrease of the platelet morphology indices MPC, MPM, and PCDW, which reflects an increase in platelet activation. In Siberian Huskies, strenuous sled-pulling activity results in a significant decrease in MPC and an increase in the ability of platelets to be activated by phorbol myristate acetate; however, secondary and tertiary hemostasis (ie, the activity of physiologic inhibitors of coagulation and fibrinolysis) were not assessed in that study.

On the basis of the results in humans, it can be hypothesized that submaximal aerobic exercise may be associated with changes in platelet morphology indices and platelet function as well as secondary and tertiary hemostasis. Therefore, the purpose of the study reported here was to investigate the influence of standardized submaximal moderate exercise on primary, secondary, and tertiary hemostasis in dogs. The plasma lactate concentration also was assessed to ensure that the degree of exercise was indeed submaximal (ie, aerobic).

### Materials and Methods

#### Animals

Nine healthy Beagles (5 neutered males and 4 spayed females) with a median age of 4 years (range, 2 to 4 years) and a median body weight of 14 kg (range, 10.5 to 18.2 kg) were used in the study. The dogs were assessed as healthy on the basis of results of physical examination, a CBC, and serum biochemical analysis, echocardiography, and evaluation of fructosamine concentrations. The prospective investigation was approved by the Ethics Committee for Animal Welfare, Regional Board Giessen, Germany.

#### Study design

The same measurements were performed in each dog to obtain an intraindividual and an interindividually comparative analysis of the impact of standardized submaximal exercise on the coagulation system. In this study, each dog served as its own control animal.

Several weeks before the start of the study, the dogs were carefully acclimatized to exercising on a treadmill to avoid any influence of excitement or anxiety on the results. The dogs were included in the study only when they were appropriately conditioned to run on the treadmill; however, they were not specifically trained to achieve a certain fitness level. Exercise was always performed between 8:00 AM and 2:00 PM to avoid possible circadian effects on variables.

#### Catheter insertion

Thirty minutes before a dog began exercising, a 16-gauge indwelling polyurethane catheter was placed in a jugular vein by use of the catheter-through-the-needle method via a 14-gauge cannula provided by the manufacturer, as described elsewhere. The catheter was inserted at this time to avoid excitement attributable to catheterization during the study period. Because the jugular catheter was held in position with an elastic bandage, a T-shaped stopcock with extension was attached to the catheter to facilitate sample collection.

#### Submaximal moderate exercise

In all dogs, exercise was performed at ambient temperature (22°C). Food was withheld for 12 hours before the exercise period. Catheterized dogs ran on a treadmill at a velocity of 6 km/h for 13 minutes. Initial slope of the treadmill was 0°. After 3 minutes, the slope of the treadmill was increased 4°, and the slope was increased an additional 4° every 2 minutes until a slope of 20° was achieved. During the exercise period, the dogs were supervised by one of the authors (EE) and restrained with a harness and leash to ensure they ran steadily on the treadmill. Heart rate was constantly recorded by use of telemetry. Mean heart rate at each time point was calculated from the last 20 consecutive measurements prior to collection of blood samples.

#### Sample collection

Blood samples were collected 2 minutes before the exercise period, within 1 minute after the 13-minute exercise period, and at 60 minutes (ie, 47 minutes after the exercise period). For each sample, the first 5 mL of blood (slightly more than 2 times the dead space of the venous catheter [0.4 mL] and extension set [2.0 mL]) was aspirated into a 5-mL syringe and discarded to avoid sample dilution as recommended for nonheparinized saline (0.9% NaCl) solution. Blood then was aspirated into two 10-mL syringes and placed into anticoagulant-containing tubes. After sample collection, the central venous catheter was flushed with nonheparinized saline solution.

#### Plasma lactate concentration

For assessment of the plasma lactate concentration, blood samples were placed into 1.2-mL tubes that contained NaF. Plasma was separated from the erythrocytes within 15 minutes after sample collection via centrifugation at 15,000 X g for 2 minutes, and analysis was performed immediately thereafter by use of a clinical chemistry analyzer.

#### Hematologic analysis

Blood samples for hematologic analysis were placed into 1.2-mL tubes that contained EDTA. A laser-based hematology system with veterinary software was used. Hematologic analysis included a WBC count, Hct, platelet count, and platelet morphology indices.

Platelet analysis was performed via measurement of the intensity of 2-D laser light scattering. Results were then converted to approximate measures of platelet volume and the refractive index. Platelet morphology indices were measured via flow cytometry in the platelet channel; these indices included MPV, MPC (measurement of platelet density calculated directly from the refractive index), MPM (calculated from the platelet dry mass histogram [MPV X platelet content/100]), and PCDW (measure of the variation for change in platelet shape [MPC X 100/SD of the MPC]).
Whole blood aggregometry—Blood samples were placed into 4.5-mL collection tubes that contained the thrombin inhibitor hirudin at a concentration of 25 µg/mL, and platelets were allowed to sit at 22°C for 30 minutes prior to analysis. Whole blood aggregometry was performed by use of an impedance-based platelet function analyzer. Aggregometry was performed automatically with single-use test cells consisting of 2 incorporated sensor units, each with 2 metal electrodes. Aggregation evaluation was performed with hirudin-anticoagulated whole blood in accordance with the manufacturer's instructions. Briefly, 300 µL of isotonic saline solution heated to 37°C was pipetted into each test cell. Then, 300 µL of whole blood anticoagulated with hirudin was added; thus, a 1:2 dilution was achieved. Samples were allowed to incubate (with stirring) for 3 minutes at 37°C; measurements then were initiated by adding 20 µL of collagen, which was used at 4 final concentrations (1.6, 3.2, 5, and 10 µg/mL). Aggregation was also evaluated without any agonist to assess spontaneous aggregate formation.

The change in impedance caused by the adhesion and aggregation of platelets on the electrodes was monitored continuously. In contrast to the standard test time of 6 minutes for human blood, a recording time of 20 minutes was used for the canine blood, which allowed the curves to plateau. During the analysis, the sample-reagent mixture was stirred at 800 revolutions/min.

Measurements were always performed in duplicate. The mean was calculated automatically by the analyzer, and results were provided as the number of AUs, which reflected the increase in impedance during the measurement. Results automatically reported by the analyzer included the maximum aggregation (in AUs) and the velocity (in AUs/min [ie, the maximum increase of the aggregation curve]). An electrical internal quality-control assessment was performed once each day.

Secondary and tertiary hemostasis—Whole blood placed into 10-mL silicone-lined evacuated tubes that contained trisodium citrate (0.106 mol/L) was used for characterization of secondary and tertiary hemostasis. The tubes for coagulation testing were incubated carefully several times to ensure mixing of citrate and blood. The ratio of whole blood to anticoagulant was 9:1 (vol/vol). Collection tubes were carefully inspected to ensure proper filling; only tubes with a ratio of 9:1 for blood to citrate anticoagulant were included in the study.

Citrated whole blood was centrifuged at 850 × g for 10 minutes within 1 hour after sample collection. Citrated plasma was separated from the erythrocytes and centrifuged again at 850 × g for 10 minutes to remove nonsedimented platelets prior to freezing, as recommended. Supernatant was removed and stored at −80°C until analysis. Analysis was performed within 16 days after sample collection. For all analytes, sample stability was > 12 months. Immediately before analysis, plasma samples were thawed at 37°C in a water bath, as recommended elsewhere, to ensure the cryoprecipitate was completely dissolved. Samples then were centrifuged again at 850 × g for 10 minutes to ensure that the platelet needed.
In case of a nonnormal distribution (ie, number of large platelets), logarithmic transformation of data was performed. Because of 1 missing value at 60 minutes, the differences between results obtained at the 3 sampling time points were assessed for hematologic analyses, lactate concentration, and the APC ratio via a nonparametric Wald test. Exercise-induced changes in whole blood aggregometry were assessed with an ANOVA and covariance after logarithmic transformation of data. Covariates were the collagen concentration as well as the interaction between exercise (ie, the time point of sample collection) and collagen concentration. All analyses were performed with commercially available statistical software.* Values of \(P < 0.002\) after Bonferroni correction were considered significant for all hematologic analyses, lactate concentration, and the APC ratio.

**Results**

All dogs completed the 13-minute run without any difficulties. Submaximal exercise resulted in a significant (\(P < 0.001\)) increase in heart rate from a median value of 102 beats/min (range, 86 to 132 beats/min) at time 0 to 192 beats/min (range, 131 to 209 beats/min) at 13 minutes (immediately after exercise; Figure 1; Table 1). Heart rate returned to baseline values (median, 92 beats/min; range, 63 to 135 beats/min) at 60 minutes. There was no significant change in lactate plasma concentration immediately after exercise. Exercise induced a slight but nonsignificant increase in Hct from a median value of 0.42 L/L at time 0 to a median of 0.44 L/L at 13 minutes. The leukocyte count and the number of platelets at 13 minutes were not significantly different from the values at time 0.

![Figure 1](image)

**Table 1**—Influence of submaximal aerobic exercise in 9 Beagles on heart rate, lactate concentration, CBC results, and platelet activation indices.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference interval</th>
<th>0 minutes</th>
<th>13 minutes</th>
<th>60 minutes</th>
<th>(P) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>60–125</td>
<td>103 ± 15</td>
<td>183 ± 28</td>
<td>94 ± 21</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>0.51–2.49</td>
<td>0.66 ± 0.20</td>
<td>0.88 ± 0.40</td>
<td>0.70 ± 0.19</td>
<td>0.078</td>
</tr>
<tr>
<td>Hct (L/L)</td>
<td>0.39–0.56</td>
<td>0.42 ± 0.03</td>
<td>0.42 ± 0.03</td>
<td>0.43 ± 0.05</td>
<td>0.133</td>
</tr>
<tr>
<td>WBCs ((\times 10^9) cells/L)</td>
<td>5.40–13.70</td>
<td>6.30 ± 1.70</td>
<td>8.30 ± 1.70</td>
<td>8.90 ± 1.50</td>
<td>0.452</td>
</tr>
<tr>
<td>Platelets ((\times 10^{12}) platelets/L)</td>
<td>172–610</td>
<td>315 ± 55</td>
<td>297 ± 84</td>
<td>298 ± 62</td>
<td>0.385</td>
</tr>
<tr>
<td>MPC (g/dL)</td>
<td>15.20–20.58</td>
<td>20.10 ± 2.40</td>
<td>22.10 ± 1.90</td>
<td>21.20 ± 2.20</td>
<td>0.008</td>
</tr>
<tr>
<td>PCDW (g/dL)</td>
<td>2.88–6.23</td>
<td>6.90 ± 1.00</td>
<td>6.20 ± 1.20</td>
<td>6.20 ± 0.80</td>
<td>0.013</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>8.5–13.6</td>
<td>11.8 ± 1.9</td>
<td>10.4 ± 1.5</td>
<td>10.3 ± 1.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No. of large platelets</td>
<td>3–52</td>
<td>28 (4–72)</td>
<td>9 (2–63)</td>
<td>23 (4–42)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MPM (pg)</td>
<td>1.4–2.0</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>0.363</td>
</tr>
</tbody>
</table>

Values reported are mean ± SD, except for number of large platelets, which are reported as median (range). Time 0 was immediately before the exercise period, 13 minutes was the end of the submaximal exercise period, and 60 minutes was after a period of recovery from exercise.

*Values of \(P < 0.002\) after Bonferroni correction were considered significant.
Submaximal exercise did not have a significant impact on MPC and PCDW. There was a significant ($P < 0.001$) exercise-induced decrease in MPV from a median value of 12.3 fL at time 0 to 10.6 fL at 13 minutes, and the number of large platelets decreased significantly ($P < 0.001$) from a median of 28 (range, 4 to 72) at time 0 to 9 (range, 2 to 63) at 13 minutes.

Moderate exercise did not have a significant impact on platelet function, as reflected by the maximal aggregation and velocity of aggregation (Figure 2; Tables 2 and 3). The 2-way ANOVA revealed a significant ($P < 0.001$) influence of agonist concentration on the maximal aggregation and velocity of aggregation.

The TEG variables did not change significantly immediately after exercise (Table 4). Similarly, there were no significant changes immediately after exercise in any of the coagulation variables reflecting secondary and tertiary hemostasis (Table 5).

**Discussion**

To the authors' knowledge, the study reported here was the first in which investigators evaluated the impact of exercise on platelet aggregation, secondary and tertiary hemostasis, and TEG in dogs. Overall, submaximal exercise did not induce any major changes in coagulation variables, except for a significant decrease in the number of large platelets and MPV. Because platelet activation is accompanied by fluid uptake and thus an increase in MPV, the decreased number of large platelets and MPV immediately after exercise may be indicative of a decrease in platelet activation status.

It can be hypothesized that the decrease of MPV after exercise in the present study was attributable to formation of microaggregates of large platelets, thus resulting in a decrease in the number of large platelets and consumption of the most active platelets. Theoretically, exercise may induce platelet microaggregate formation mediated through shear stress and the plasma epinephrine concentration accompanying physical activity. In humans, shear stress and epinephrine can activate platelets synergistically through von Willebrand factor interaction with glycoprotein Ib. Platelet activation...
is followed by degranulation, transient aggregate formation mediated by von Willebrand factor, and finally deaggregation resulting in recirculation of exhausted defective platelets with secondary storage pool disease, as has been reported for humans.29

Interestingly, the lack of significant exercise-induced changes in MPC and PCDW in the study reported here was in contrast to results of another study12 in dogs in which investigators detected a decrease in MPC and PCDW suggestive of platelet activation after short-duration strenuous sled-pulling activity. A similar result was seen in human athletes after they finished running a marathon.29 However, in contrast to the present study, the effect of strenuous (rather than moderate) exercise was evaluated in those other 2 studies that resulted in leukocytosis10 and activation of neutrophils,12 most likely because of an inflammatory reaction caused by exercise-induced tissue damage.29 In the dogs of the present study, there was no evidence of an inflammatory reaction, which is a probable explanation for the differences between the present study and the previous studies. Theoretically, breed-specific differences in platelet function may have contributed, but to the authors’ knowledge, breed-specific platelet function has not been reported for Beagles and Siberian Huskies.

Although submaximal aerobic physical activity in dogs did not result in significant changes in platelet function as assessed by aggregometry, human platelets had markedly increased sensitivity to collagen-induced aggregation in amateur runners taking part in a marathon race.29 However, there is controversy about platelet function in humans following strenuous exercise because markedly decreased platelet function has been detected in marathon runners immediately after a race and 24 hours after finishing strenuous physical activity.30 A limitation of the present study in dogs and most studies in humans is the fact that only 1 exercise protocol has been investigated, which results in conflicting opinions in the literature regarding the beneficial or potentially unfavorable effect of physical activity on the coagulation system. Rarely, platelet adhesiveness and aggregability have been evaluated in humans performing exercise of various intensities. The authors of 1 study31 reported that platelet adhesiveness and aggregability may be sensitized by strenuous exercise in healthy subjects and patients with coronary heart disease, whereas moderate exercise is followed by a significant decrease in platelet function in healthy volunteers and a pattern of platelet hypofunction in patients. These effects typically are more pronounced in sedentary people than in active people.31 Those authors concluded that moderate exercise may prevent thromboembolism, but strenuous exercise is associated with thrombophilia.32 A possible reason for the reduced platelet function detected for moderate-intensity exercise is the fact that lower concentrations of catecholamines and a higher amount of nitrous oxide are released from vascular endothelial cells,32 and this release can inhibit thrombus formation under high shear stress and decrease the agonist-induced upregulation of P-selectin, a platelet activation marker, and glycoprotein IIb/IIIa, the fibrinogen receptor in platelets.33,34

The TEG variables were not influenced by moderate physical activity in the dogs of the present study reported here. This is in contrast to results in humans, who had an increase of the maximum clot firmness (comparable with MA) following a 42,195-m downhill race.4

Similarly, moderate exercise did not influence secondary and tertiary hemostasis in dogs of the present study. However, a marked activation of coagulation reflected by aPTT, OSPT, and the FVIII concentration as well as an increase in the concentrations of natural in-

| Table 4—Influence of submaximal aerobic exercise in 9 Beagles on secondary and tertiary hemostasis. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable        | Reference interval | 0 minutes | 13 minutes | 60 minutes | P value* |
| Angle (°)       | 36.9–74.6         | 36.0       | 75 ± 7     | 71 ± 13     | 0.430 |
| K (min)         | 1.3–5.7           | 5.6        | 8.2 ± 1.7  | 6.0 ± 1.8   | 0.120 |
| Protein C (%)   | 76–119            | 77 ± 15    | 75 ± 7     | 71 ± 13     | 0.430 |
| Protein S (%)   | 74–161            | 97 ± 16    | 95 ± 17    | 89 ± 11     | 0.093 |
| APC ratio       | 2.0–3.0           | 2.4 ± 0.1  | 2.6 ± 0.1  | 2.4 ± 0.2†  | 0.043 |
| D-dimer (mg/mL) | 0.023–0.65        | 0.23 ± 0.03| 0.23 ± 0.04| 0.23 ± 0.01 | 0.586 |

1At 60 minutes, 1 outlier exceeded 3 times the SD and was removed. See Table 1 for remainder of key.

| Table 5—Influence of submaximal aerobic exercise in 9 Beagles on secondary and tertiary hemostasis as assessed via TEG. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable        | Reference interval | 0 minutes | 13 minutes | 60 minutes | P value* |
| R (min)         | 1.8–8.6          | 6.2 ± 1.7     | 7.3 ± 2.2    | 6.0 ± 1.8    | 0.120 |
| K (min)         | 1.3–5.7          | 5.6 ± 1.9     | 5.0 ± 2.2    | 4.5 ± 2.3    | 0.053 |
| Angle α (°)     | 36.0–74.6        | 36.0 ± 11.1   | 41.3 ± 11.4 | 43.1 ± 9.3   | 0.368 |
| MA (mm)         | 42.9–67.9        | 46.0 ± 11.0   | 48.0 ± 5.0   | 52.0 ± 8.0   | 0.521 |
| G (dynes/cm²)   | 3.2–9.8          | 5.6 ± 2.0     | 4.7 ± 1.0    | 5.7 ± 2.0    | 0.465 |

Angle α = Indicative of the rapidity of fibrin cross-linking. G = Clot stability (mainly reflecting platelet aggregation mediated by von Willebrand factor, and finally deaggregation resulting in recirculation of exhausted defective platelets with secondary storage pool disease, as has been reported for humans.29

Interestingly, the lack of significant exercise-induced changes in MPC and PCDW in the study reported here was in contrast to results of another study12 in dogs in which investigators detected a decrease in MPC and PCDW suggestive of platelet activation after short-duration strenuous sled-pulling activity. A similar result was seen in human athletes after they finished running a marathon.29 However, in contrast to the present study, the effect of strenuous (rather than moderate) exercise was evaluated in those other 2 studies that resulted in leukocytosis10 and activation of neutrophils,12 most likely because of an inflammatory reaction caused by exercise-induced tissue damage.29 In the dogs of the present study, there was no evidence of an inflammatory reaction, which is a probable explanation for the differences between the present study and the previous studies. Theoretically, breed-specific differences in platelet function may have contributed, but to the authors’ knowledge, breed-specific platelet function has not been reported for Beagles and Siberian Huskies.

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The TEG variables were not influenced by moderate physical activity in the dogs of the present study reported here. This is in contrast to results in humans, who had an increase of the maximum clot firmness (comparable with MA) following a 42,195-m downhill race.4

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1At 60 minutes, 1 outlier exceeded 3 times the SD and was removed. See Table 1 for remainder of key.
hibitors of coagulation, including protein C and protein S, and evidence of fibrinolysis indicated by an increase in D-dimer concentration has been detected in humans after strenuous exercise.6 A probable reason for the lack of major changes in coagulation variables in the present study in dogs, compared with results for the studies in humans, is the fact that coagulation evaluations in healthy humans have primarily been conducted in athletes performing strenuous exercise, such as a marathon race, which is much more exhausting than the moderate exercise performed by the dogs in the study reported here. Muscular microtrauma is considered to be responsible for activation of the coagulation system1; thus, it can be hypothesized that activation of coagulation will increase with increases in the severity of physical exercise. This hypothesis is supported by a review article,3 the author of which analyzed the results of research in humans regarding the effects and the underlying mechanisms of exercise on coagulation. On the basis of the reviewed literature, that author reported that light acute exercise does not affect platelet reactivity and coagulation and increases fibrinolytic activity, whereas moderate acute exercise results in suppressed platelet reactivity and enhanced fibrinolysis, and strenuous acute exercise increases platelet reactivity, coagulation activity, and fibrinolysis.39 Thus, moderate exercise is considered to be safe and effective for minimizing the risk of cardiovascular diseases in humans by inducing beneficial antithrombotic changes.35

Results for the dogs of the present study are comparable to those described for humans performing light exercise. It must be mentioned that the gradation of exercise intensity in humans was based on the maximum oxygen consumption (ie, reflecting the functional aerobic work capacity of a subject).38 Because the calculation of maximum oxygen consumption is performed on the basis of cardiac output and arterial and venous oxygen concentrations, it cannot be easily determined in exercising dogs and therefore was not measured in the study reported here.

In the present study, intensity of exercise was reflected by the lactate plasma concentration. Because the lactate concentration immediately after exercise was not significantly different from the concentration at time 0 and remained within the reference interval, it can be concluded that exercise was aerobic and thus submaximal as intended; however, a differentiation between light and moderate exercise could not be made.

Regarding the methods used in the present study, submaximal aerobic exercise was chosen because pet dogs are rarely expected to perform maximal exercise (ie, physical activity exceeding the aerobic threshold); thus, the present study is likely to provide clinically useful data for most pet dogs evaluated at veterinary practices and clinics.

Sample acquisition was performed through a 16-gauge central venous catheter inserted via the catheter-through-the-needle technique to provide rapid sample collection at specific time points and to avoid painful stimuli to or excitement of the dogs that might have influenced the results. Sample collection through venous catheters may theoretically induce shear stress and thus changes in the coagulation pattern. However, a sampling-induced influence of coagulation was ruled out in another study40 for a venous catheter that was similar to the one used in the present study. Sample dilution resulting from flushing of the catheter was avoided by discarding a 2-fold volume of the dead space of the catheter and extension set as has been recommended for sample collection in humans.37 Direct aspiration of samples into evacuated tubes may have further increased the likelihood of causing platelet activation via shear stress.37 However, samples in the present study were collected in a plastic syringe and then placed into citrated tubes to reduce shear stress caused by an evacuated tube system.

A novel impedance-based whole blood aggregometer was chosen to assess platelet function because of the fact that light transmission aggregometry (Born method), which is the most commonly used method for platelet function testing, has some drawbacks. Disadvantages of the Born method include the need for preparation of platelet-rich plasma, which results in separation of other blood cells from platelets, which can influence platelet function38,39; the loss of platelets during the process of preparation as has been reported in humans40-42; and the fact that giant platelets, which may be hypoactive or hyperactive, are commonly not included in platelet-rich plasma.43

The impedance aggregometer was used in the present study to determine platelet function in diluted whole blood by use of disposable test cells with duplicate impedance sensors. Thus, possible sources of error (eg, cleaning of electrodes between analyses that was required in older impedance aggregometers) were avoided.38 In the present study, the thrombin antagonist hirudin was used as an anticoagulant for canine blood because it preserves the physiologic concentrations of ionized calcium and magnesium better than citrate does.44 In a previous study44 and another study22 conducted by our research group, the lowest intra-assay coefficient of variation was observed for a collagen concentration of 10 mmol/L; therefore, this concentration of agonist was chosen as the starting concentration. When interpreting the data, it must be considered that previous investigators in that other study44 found that the lowest interindividual variation was for collagen concentrations between 3 and 5 mmol/L.

Regarding the preparation of citrated plasma, it must be mentioned that the centrifugation routinely used in the authors’ laboratory was < 1,500 × g (ie, the force recommended by the Clinical and Laboratory Standards Institute).57 However, according to recommendations by the Clinical and Laboratory Standards Institute, alternative times and forces may be used providing the plasma platelet count is ≤ 10 × 10⁹ platelets/L,58 which has been achieved for the protocol used in the present study. For human laboratories, a variation in centrifugation force has been reported, ranging from 500 × g to 3,000 × g.46 A limitation of the present study was the fact that the number of dogs examined was comparatively low, so significant effects may not have been detected.

On the basis of analysis of results of the present study, it can be concluded that submaximal aerobic ex-
ercise was not associated with a thromboembolic risk in healthy dogs. It might be of interest in a future study to evaluate whether there is an impact of the degree of exercise (walking vs submaximal or maximal exercise) on platelet activation and whether physical activity is associated with an increased risk of thromboembolism in dogs already in a hypercoagulable state.

References


